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APPLICATION OF SHANNON–WIENER INDEX AS A MEASURE OF POLLUTION OF RIVER GANGA AT PATNA, BIHAR, INDIA

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ABSTRACT

Monthly variations of the Shannon–Wiener species diversity index ($\bar{H}$) for benthic macroinvertebrates were recorded at three stations on river Ganga at Patna from July 1985 to June 1986. The Shannon–Wiener index is found to vary from 0.346 to 1.238 at station III, and indicates severe environmental stress. The range at station II, 0.689 to 2.434, is indicative of an intermediate state of environmental pollution, and the range at station I, 0.798 to 2.608, reflects a comparatively low load of pollutants.

INTRODUCTION

While many investigations have been conducted to assess the pollution of the Ganga[1–4], hardly any has adopted a quantitative approach through which one can measure the intensity of pollution and compare the degree of pollution between two sections of a river or that of one stream with that of another. The studies on the river Khan[5] and the Ganga[3,6] are perhaps the closest to a somewhat quantitative approach. Of the numerous proposed quantitative indices of pollution[7–10], the index based on the information theory of Shannon and Wiener[11,12] is extensively used in the USA[7–9,13,14] and UK[15–19] to assess environmental perturbations of fluviatile systems.

The Shannon–Wiener species diversity index is defined as

$$\bar{H} = -\sum_{i=1}^{S}\left(\frac{n_i}{N}\right)\log_2\left(\frac{n_i}{N}\right),$$

where S is the number of species in a sample, N the total number of individuals in the sample, and n_i the number of individuals of each species, i.e. $N = \Sigma n_i$.

The concept of species diversity is based on the theory that an aquatic biotal community inhabiting an environment that is free from pollution is characterized by the occurrence of a wide variety of species but only by a moderate number of individuals of each species. A change in the biotal community structure, resulting in less species but greater abundance of select tolerant ones, reflects the advent of a condition of environmental stress. In fact environmental perturbation in a lotic aquatic ecosystem can be quantitatively evaluated by delineation of autochthonous macroinvertebrate communities through biological indices that summarize a vast amount of hydrobiological and physico-chemical environmental information in a meaningful manner. The same cannot be said of planktonic communities in a fluviatile environment since such biota can be exochthonous in origin despite being detected *in situ* even in intensely polluted zones.

Assessment of water quality in terms of numerical indices has been mostly attempted in river and stream pollution studies[20]. Perhaps benthic macroinvertebrates make an ideal subject for such studies because of their habitat preference and limited motility and, hence, have often been used for biological assessment of water quality[21].

The close relationship obtaining between species Shannon–Wiener diversity index, $\bar{H}$, and pollutional levels[21] is as follows:

Shannon–Wiener species diversity index ($\bar{H}$)	Water condition
> 3	Clean
1–3	Moderately polluted
< 1	Heavily polluted

---

*For correspondence.

There exists a slightly different scale[22] in terms of species diversity ($\bar{H}$), which is 3.0–4.5, 2–3, 1–2, 0–1, the degrees of pollution being slight, light, moderate and heavy respectively, also indicating a negative correlation between the Shannon–Wiener index and pollution level.

METHODS

The annual cycle of species diversity among benthic macroinvertebrates was studied at three stations on river Ganga at Patna from July 1985 to June 1986. The city of Patna, situated on the right bank of the Ganga, is located at 25° 37′ N and 85° 21′ E. The three stations selected for observations were designated stations I, II and III (figure 1). The distances between stations I and II, and between II and III are 2.4 km and 3.2 km respectively. The observation stations were located about 100 m downstream from bathing centres (called 'Ghat' in local parlance) and also from discharge points of effluents, of which there are four, two slightly upstream of station I and one each slightly upstream of stations II and III.

Twice-a-month collections were made with an Ekman dredge covering an area 225 cm^2 of the substratum. The material collected was sifted through a No. 40 sieve and the organisms were collected and identified. The two samples of a month were pooled to constitute monthly collection, and all the 24 of the year grouped to determine the annual value of the Shannon–Wiener index.

RESULTS AND DISCUSSION

The macroinvertebrates encountered are listed in table 1. Figure 2 presents the changes in the Shannon–Wiener index during the period of study.

The derived annual mean monthly $\bar{H}$ values are: 1.394, 1.333 and 0.754 at stations I, II and III respectively. The three stations do not show a very significant difference in the means, though the mean monthly species diversity value of station III is much less compared to those of the other two stations. The Shannon–Wiener index values show the pollution load at each station reflected by the diversity of benthic macroinvertebrates. In the present study, the Shannon–Wiener index was found to vary from 0.346 to 1.238 at station III, which is indicative of severe environmental stress, while the range at station II, 0.689 to 2.434, is indicative of an intermediate state of environmental pollution, and the range at station I, 0.798 to 2.608, indicates a comparatively low load of pollutants. The wide range of fluctuation of the monthly Shannon–Wiener diversity index of benthic macroinvertebrates during the study period needs explanation.

The index was at its lowest at station I (0.798) in January 1986; at station II (0.689) in October 1985; and at station III (0.346) in September 1985. This is attributable to possible annihilation of the benthic fauna, probably caused by the additive effects of the high current of snow-melt waters from the Himalayas, added to all along by tributaries originating from the Himalayas in the north, and of toxicants. A

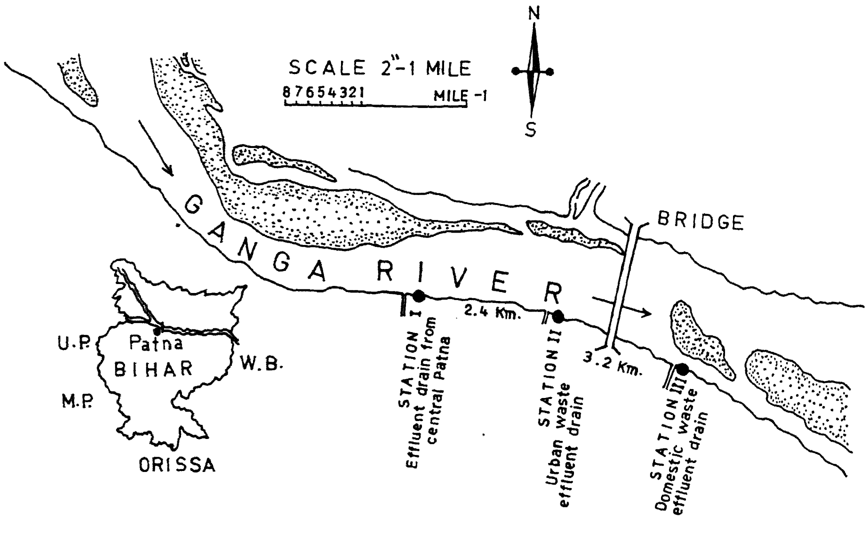

Figure 1. Location map of the three sampling stations (I, II and III) on river Ganga at Patna.

Table 1 *List of benthic macroinvertebrates collected from river Ganga at Patna*

Species	Station I	Station II	Station III
Polychaeta			
Namalycastis indica	+	+	−
Oligochaeta			
Tubifex sp.	−	+	+
Nais simplex	−	−	+
Limnodrillus hoffmeisteri	−	−	+
Insecta			
Chironomus sp.	−	+	+
Dragonfly nymph	+	+	+
Pelecypoda			
Lemellidens consorinus (Lea)	+	+	−
Lemellidens marginalis	+	−	−
Parreysia (Parreysia) *corrugata* var. *nagpoorensis* (Lea)	+	+	−
Indonia coerulea (Lea)	+	+	−
Corbicula striatella Deshayes	+	+	−
Gastropoda			
Digoniostoma pulchella (Benson)	+	+	−
Viviparus bengalensis (Lamarck)	−	+	−
Glessula sp.	+	+	+
Melania striatella tuberculata (Muller)	+	+	−
Melania (Plotia) *scabra* (Muller)	+	+	+
Melania scabra var. *elegans*	+	+	−
Lymnea sp.	+	−	+
Total	13	14	8

+, Present; −, Absent.

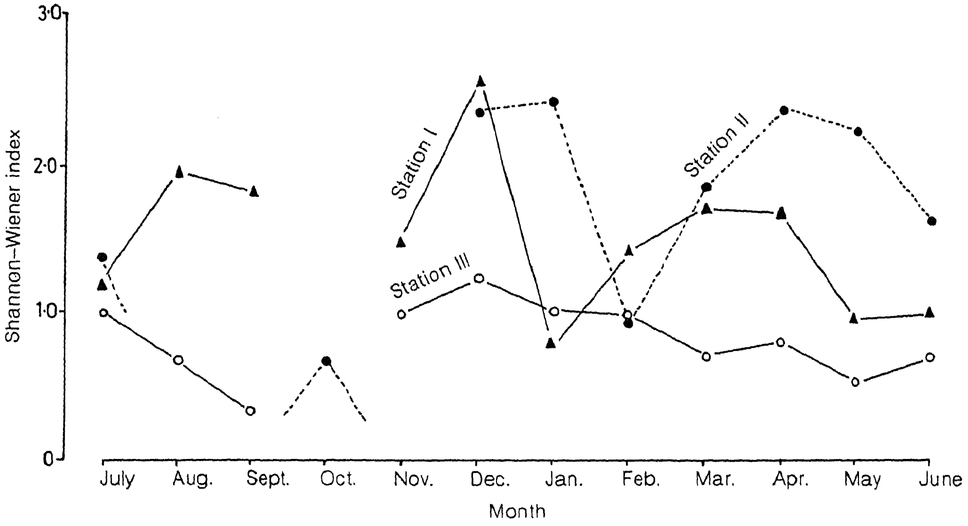

Figure 2. Monthly variation in Shannon–Wiener index ($\bar{H}$) values at stations I, II and III from July 1985 to June 1986.

similar observation was recorded in Keystone reservoir in the USA[23]. The somewhat lower values of the index during other months, viz. May 1986 at station I, February 1986 at station II, and August 1985 and February to June 1986 at station III, are probably attributable to the continued residual effect of the pollutants settled at the bottom leading to reduced diversity of benthic macroinvertebrates. The diversity index is zero where macroinvertebrates are totally absent, having been annihilated by the heavy pollution load. The high value of the diversity index at station I (2.608) in December 1985, at station II (2.434) in January 1986, and at station III (1.238) in December 1985 might be due to a reduced discharge of effluents into the river, as well as to low (winter) temperature and consequent decrease in the amount of organic matter, resulting in greater diversity of the benthic macroinvertebrates. Exposed to progressively increasing amount of domestic discharge and urban runoff, the benthos at station III is found to be less able to support a diverse and stable macrobenthic community, and a state of maximum pollution prevails. The present study shows that the species distribution patterns are indicative of the sum total of environmental perturbations, quite naturally resulting in reduction in the number of the sensitive species and a phenomenal increase in the number of the tolerant ones. This could be depicted in a species distribution pattern[24]. Monsoonal inundations, which dilute the pollution, the snow-melt waters in summer from the Himalayas, the managerial policy of waste release, the ambient temperature of the receiving water, and errors of random sampling together constitute a complex system. The diversity of benthic macroinvertebrates reacts to the net result of pollutional load. The relatively constant values of the Shannon–Wiener index at the farthest downstream-placed station, 5.6 km downstream of Patna, reflects a relatively stabilized level of pollution from all effluent points, taking all reacting and interacting factors into account. The varying values of the index in different months at the other stations reflect a variable pollutional load from individual effluent drains interacting with abating forces like dilution of water from various sources.

CONCLUSION

The Shannon–Wiener species diversity index ($\bar{H}$) can be of utility for the evaluation of the stresses to the benthic macroinvertebrate populations due to changes in water quality. It can serve also as a direct indication of the cumulative effect of environmenta stresses on benthic organisms.

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EFFECT OF EXOGENOUS 17β-ESTRADIOL ON GONADAL PHOTOSTIMULATION IN HOUSE SPARROW, *PASSER DOMESTICUS* (LINN.)

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ABSTRACT

Intramuscular injection of 17β-estradiol inhibited photoperiod induction of gonads in female subtropical house sparrow (*Passer domesticus*), with no effect on body weight. This suggests that exogenous 17β-estradiol has an antigonadal effect in this species. The effect is due to the negative feedback mechanism of the hormone at the hypothalamo–hypophyseal axis.

INTRODUCTION

It is well established that sex steroids play an important role in the regulation of reproductive and associated functions in birds. Different gonadal responses to exogenous androgen and estrogen have been reported, which have antigonadal, progonadal, or no effect in several avian species[1–8]. The effect of exogenous gonadal steroids has been less extensively studied in subtropical photoperiodic bird species, particularly in females. Both progonadal and antigonadal effects of testosterone have been reported in temperate zone species of the house sparrow[5]. In view of the paucity of a unifying concept, we investigated the effect of exogenous 17β-estradiol on the regulation of ovarian, oviducal and body weight responses in the house sparrow, a subtropical, non-migratory passerine bird.

MATERIALS AND METHODS

Adult female house sparrows were captured locally and acclimatized for 15 days to laboratory conditions. They were then exposed to an 8L:16D photoperiod regime for eight weeks to make them photosensitive. At this time all the birds had regressed ovaries (ovarian weight 4–6 mg) and normal body weight (18–21 g). In the house sparrow, long days are gonadostimulatory and short days (light for less than 11 h) are non-stimulatory[9]. Food and water were given *ad libitum*. The bird enclosure was illuminated by 20-watt fluorescent tubes, which provided a light intensity of about 400 lux at perch level. At each laparotomy, ovarian weight was assessed by comparing the size of the ovary *in situ* with a standard set of ovaries of known weight. The error by this method was less than ±20%. Data were analysed statistically by the Student's t test[10].

Experiments were performed, viz. with photosensitive birds and with photostimulated birds.

Photosensitive birds (exposed to short-day regime) were divided into four groups ($n = 15$ each) and exposed to long-day photoperiod (15L:9D) for 50 days. Their initial ovarian condition *in situ* (by laparotomy) and body weight were recorded. Four birds were weighed and then sacrificed; their ovaries and oviducts were taken out, freed of extra tissues, and weighed after the luminal fluid was squeezed out gently on a folded filter paper. Seven intramuscular injections of 17β-estradiol (Sigma Chemical Co., USA) were given at every two days from the day of commencement of long-day photocycle. Three doses of hormone in 0.1 ml of propyleneglycol were administered. One group received a high dose (100 μg), another received 25 μg and the third group received a low dose of 5 μg. The fourth (control) group was given only 0.1 ml propyleneglycol. Injections were given between 10.00 and 10.30 a.m. Ovarian, oviducal and body weights of three birds from each group were recorded on days 15, 30 and 50.

In the other experiment birds kept under long photoperiod regime for 40 days were used. The photostimulated birds were divided into four groups ($n = 4$–6) and exposed to the same long photoperiod regime (15L:9D) for 50 days. The birds were treated as described above for photosensitive birds, and ovarian weights were recorded on days 15, 30 and 50.

RESULTS

The results of the experiment with photosensitive birds are given in table 1.

On day 15 no differences were evident between the groups. On day 30 ovarian weight of birds given 25 and 100 μg estradiol were significantly reduced. On

Gonadal inhibition :

Table 1 *Effect of 17β-estradiol on ovarian, oviducal and body weight responses in photosensitive house sparrow under long photoperiod (15L:9D) regime*

Parameter	Day of observation	Dose: None (control)	5 μg	25 μg	100 μg
Ovarian weight (mg)	15	6.50 ± 0.28	6.10 ± 0.22	6.30 ± 0.42	5.80 ± 0.20
	30	9.70 ± 0.16	9.40 ± 0.30	8.00 ± 0.28*	6.43 ± 0.28*
	50	23.10 ± 1.22	20.10 ± 0.44**	13.00 ± 0.36*	11.00 ± 0.33*
Oviducal weight (mg)	15	4.10 ± 0.20	117.43 ± 1.90*	136.66 ± 2.60*	192.46 ± 4.05*
	30	5.70 ± 0.44	13.83 ± 0.92*	38.56 ± 0.92*	49.93 ± 5.23*
	50	15.00 ± 0.00	10.33 ± 0.60*	10.83 ± 0.60*	11.80 ± 0.68*
Body weight (g)	15	20.33 ± 0.60	19.40 ± 0.45	19.20 ± 0.46	19.43 ± 0.83
	30	20.50 ± 0.57	19.63 ± 0.56	19.33 ± 0.44	19.13 ± 0.46
	50	19.93 ± 0.07	19.33 ± 0.35	19.53 ± 0.46	19.00 ± 0.26

All values are mean ± SEM.
Significance of difference (vs respective control): $^{*}P<0.001$, $^{**}P<0.05$.

day 50 photostimulation of ovarian weight was evident in all the four groups, but estradiol produced a dose-dependent inhibition of photostimulation, and the inhibition was significant at all three doses. The different groups did not differ in body weight. At the beginning of exposure to long photoperiod regime, oviducal weight was minimum (2 to 4 mg). On day 15 oviducal weight was very high in all three hormone-treated groups (table 1) but not in control birds. Moreover, there was a dose-dependent difference between the hormone-treated groups. Oviducal weight of the hormone-treated birds had decreased considerably by day 30, but were still higher than that of control birds. On day 50, however, control birds had higher oviducal weight than hormone-treated birds.

The results of the experiment with photostimulated birds are given in table 2. The dose-dependent inhibitory effect of estradiol on photostimulation of ovarian weight is again evident.

DISCUSSION

The results clearly indicate that 17β-estra treatment inhibits ovarian response in both ph sensitive and photostimulated birds under stimula long photoperiod regime (15L:9D). It is sugge that the long-day-induced ovarian growth is dir under the control of hypophyseal gonadotrop and it seems quite apparent that the exoge estradiol prevents rise in the levels of both FSH LH, thereby inhibiting ovarian growth. This effe 17β-estradiol in female house sparrow is throu short-loop negative feedback mechanism. Si reports are available for other birds[1,3,8,11]. T *et al.*[5] reported that 500 μg testosterone per (released from testosterone-filled silastic cap administered into the peritoneal cavity) stimu testicular growth in house sparrows exposed non-stimulatory (8L:16D) photoperiod. Furth dose-dependent, differential effect of testosteron

Table 2 *Effect of 17β-estradiol on long-day-induced ovarian weight responses in photostimulated house sparrow*

Parameter	Day of observation	Dose: None (control)	5 μg	25 μg	100 μg
Ovarian weight (mg)	0	16.00 ± 00	15.00 ± 00	14.25 ± 0.30	15.50 ± 0.28
	15	18.80 ± 0.58	11.50 ± 0.28*	11.00 ± 0.40*	9.00 ± 0.40*
	30	22.20 ± 0.50	13.50 ± 0.28*	13.25 ± 0.47*	10.25 ± 0.47*
	50	25.20 ± 0.37	19.50 ± 0.95*	15.50 ± 0.64*	13.00 ± 0.40*

All values are mean ± SEM.
*Significant difference (vs respective control), $P<0.001$.

been reported in the male house sparrow by the same authors. In Lalmunia, it is reported that 0.5 μg and 5.0 μg testosterone suppressed testis growth during the progressive phase but produced no effect in the late progressive and/or breeding phase[7]. However, 100 μg testosterone inhibited fully developed gonads of male Lalmunia and red-headed bunting[7]. Lower doses also suppressed gonadal growth in the house sparrow[5].

An interaction between length of photoperiod and gonadal steroids during the annual reproductive cycle has also been reported to modulate gonadotrophin secretion[11-15]. A striking difference in the rate of hypophyseal output of gonadotrophin (especially LH) between male and female birds has been observed during photostimulation[15,16]. The reason for such a difference between the sexes has been attributed mainly due to the negative feedback action[3,15].

Unlike the observations with female subtropical house sparrow, a dose-dependent, differential effect of testosterone has been reported on the testes of sexually mature house sparrow[5]. The present results are, in general, contrary to those of previous investigations, in which the testosterone could exert stimulatory or inhibitory effect on avian testes[17,18].

The oviducal hypertrophy seen in hormone-treated birds is possibly due to the influence of exogenous 17β-estradiol. But control group oviducal growth was evident when the ovaries showed growth under normal photostimulation. Increase in oviducal weight in the control group clearly indicates the high endogenous level of estradiol in photostimulated birds. Similar findings have been reported in other birds[19,20]. In the hormone-injected groups, ovarian photostimulation was brought about as the effect of exogenous hormone was removed, and thus the oviduct remained under the influence of high endogenous levels of estradiol.

The results also indicate that 17β-estradiol does not play a role in body weight regulation in the house sparrow. This is similar to results for the non-migratory resident species, the Lalmunia[7].

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COMPUTER SIMULATION OF THE INTERACTION OF PROSTAGLANDINS $PGF_{2\alpha}$ AND PGB_1 WITH DIPALMITOYLPHOSPHATIDYLCHOLINE

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ABSTRACT

Computer-aided model building, using the empirical potential energy function and the energy minimization technique, was used to study the interaction of prostaglandins (PGs) $PGF_{2\alpha}$ and PGB_1 with dipalmitoylphosphatidylcholine (DPPC). $PGF_{2\alpha}$ can interact with the DPPC molecule in two different ways. In the first model (model I) 9α- and 11α-hydroxy groups could make hydrogen bonds with the phosphate oxygens and neutralize them. In the second model (model II) 15-(S)-hydroxy group makes bifurcated hydrogen bonds with the phosphate oxygens. The 9α- and 11α-hydroxy groups are free to interact with the cation (Ca^{2+}) and neutralize its positive charge. For PGB_1 model I was energetically unfavourable, and model II had energy 30.11 kcal mol^{-1} higher than model II for $PGF_{2\alpha}$. In addition to these two binding sites, both the molecules could make hydrophobic contacts with the DPPC chains and loosely embed in the membrane (model III). The binding energy in model III was higher than that in model II. The influence of these interactions on prostaglandin-induced contractile activity of smooth muscle is discussed. An explanation for the higher activity of $PGF_{2\alpha}$ compared to that of PGB_1 is offered.

INTRODUCTION

PROSTAGLANDINS (PGs) elicit contractile response in smooth muscle[1-3] and have therapeutic value. The biological effectiveness of different PGs depends on their chemical nature[1,4-7]. Although the physiological pathway of PG action is quite complex and not well understood[8], there is enough evidence for an ionophoric effect of PG on Ca^{2+} ions in the plasma membrane[9-13].

The plasma membrane is a multicomponent system of lipids and proteins organized into functional domains[14,15]. It is possible that PGs interact directly with specific sites on receptor molecules in the membrane or perturb the dynamic properties of the lipid part of the membrane, which in turn may perturb specific proteins. It has been shown[15] that chemically active molecules can trigger a cascade of events by adsorption on membranes via electrostatic or dispersive forces. Rearrangement of phospholipids in erythrocytes in the presence of small amounts of PGE_1 was noted by Manevich *et al*[16]. Interaction of several drugs (propranolol, epinephrine, norepinephrine, vitamin D and *n*-akanols) with different lipid molecules has been studied using physicochemical techniques[17-21]. The drug molecules have been shown to interact with the hydrophobic core or the polar head, to be partly immobilized between the hydrophobic core and the polar head, or to make hydrogen bonds with the phosphate groups of the lipid molecule.

The 'form' specificity in PG action depends on the initial event in their interaction with the membrane and is related to the stereochemical aspects of different PGs. Many studies have therefore been directed at understanding the three-dimensional structures of the molecules[22-34]. As a result, many structural details are available for the PGs. Several factors, such as conformation of five-membered ring[27] and orientation of α and ω chains, have been shown to be related to activity[24-34]. However, nothing definite has been said about the primary target of PG action in membranes.

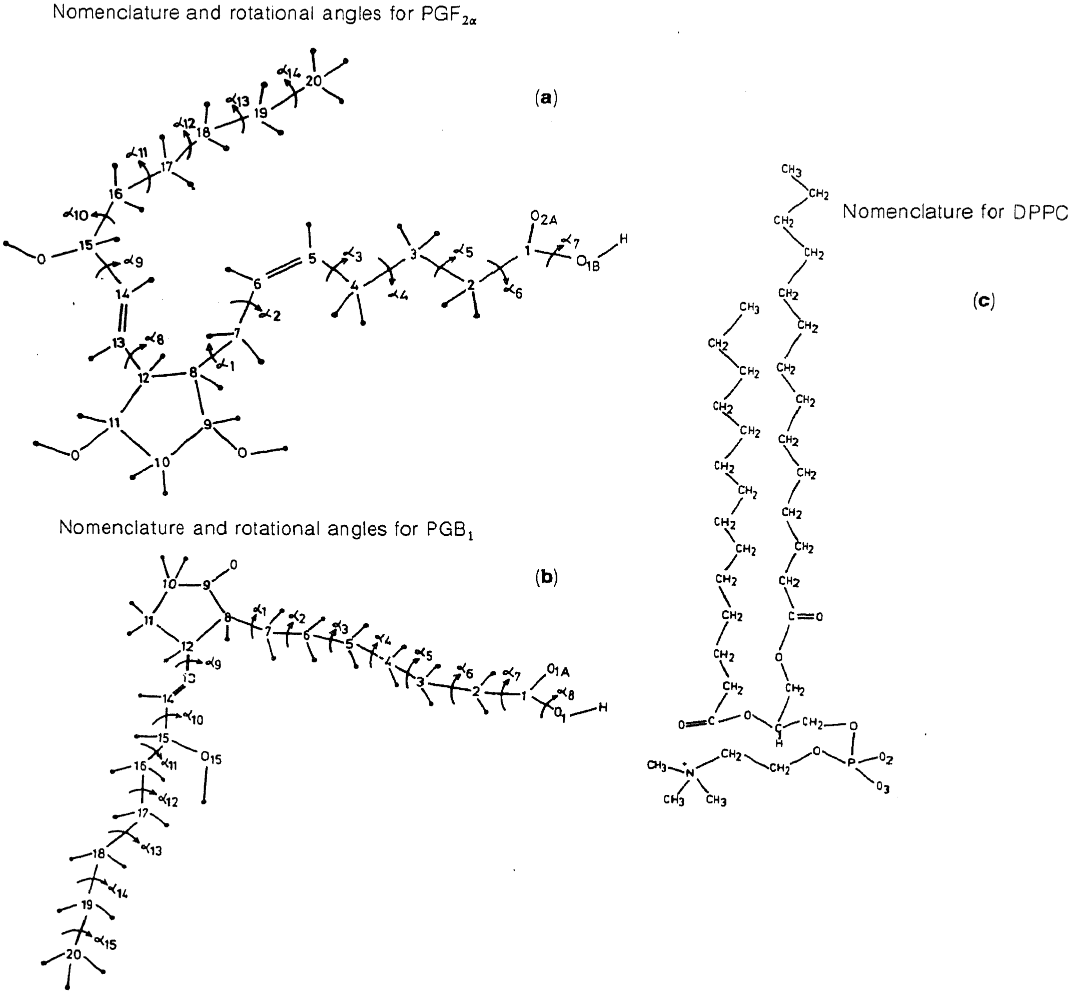
We undertook a systematic study of the interaction of PGs with membrane lipids. In earlier papers[35,36], we demonstrated that $PGF_{2\alpha}$ can bind to dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC) vesicles and cause fluidization of the membrane. There is also a reduction in the gel–liquid-crystalline-phase transition temperature. The fluidization effect is enhanced in the presence of Ca^{2+} ions[36]. These observations suggest the necessity of a detailed examination of the mode of interaction of PGs with lipid molecules.

In the present paper we describe possible models for the interaction of $PGF_{2\alpha}$ and PGB_1 with DPPC using computer modelling, allowing full (conformational and motional) freedom to the drug molecule. This technique yields, simultaneously, information regarding the stereochemical aspects of the complexes and the forces involved in the interaction.

MATERIALS AND METHODS

Initial geometry

The initial geometry of the DPPC molecule was generated from the bond length, bond angle and torsional angle data given in reference 37 on 3-lauroylpropanediol-1-phosphorylcholine monohydrate (LPPC) and by attaching fatty acyl chain to glycerol C_2. The cartesian co-ordinates of $PGF_{2\alpha}$ were generated on the basis of crystallographic co-ordinates of $PGF_{2\alpha}$ tri-*p*-bromobenzonate[22], with the difference in the orientation of the 9-OH group and the 5–6 *cis* double bond. The torsional angles given in reference 25 were incorporated in these data. Structural data for PGB_1 were generated from bond length and bond angle data given in reference 26. The torsional angles C_6–C_7–C_8–C_9 ($\alpha 1$), C_{11}–C_{12}–C_{13}–C_{14} ($\alpha 9$) and C_{13}–C_{14}–C_{15}–C_{16} ($\alpha 10$) were also fixed according to the results of a conformational study of PGB_1[31] (see figure 1a–c for nomenclature).


Nomenclature and rotational angles for $PGF_{2\alpha}$

(a)

Nomenclature and rotational angles for PGB_1

(b)

Nomenclature for DPPC

(c)

Figure 1a–c. Nomenclature for $PGF_{2\alpha}$, PGB_1 and DPPC, showing different rotational angles. The four-atom sequence for the rotational angles is described in table 2.

Potential energy function

Pair-wise (atom–atom) additive potential, comprising attractive, repulsive, electrostatic, polarization, H-bonding and torsional contributions, was used. Details are described in an earlier paper[34].

Rigid body docking of the PGs with DPPC

For the initial docking, both the molecules were considered to be rigid. The DPPC molecule was oriented such that its aliphatic chains were directed along the *z*-axis (figure 1c). The PG molecule was allowed to rotate around the DPPC molecule and translated in the *z* direction. We also allowed rotation of the PG around its own axis. Interaction energy for PG and DPPC was calculated. The energetically stable position was taken as the tentative initial binding site for further refinement. An energy constraint, viz. that for each atom–atom pair the attractive contribution should be more than the repulsive, was used for obtaining the crude models.

Refinement of the model

Refinement of the model of the complex was done in two steps. In the first step the H-bonding donors were oriented towards the phosphate oxygens. The PG molecule was allowed to translate and rotate along the H-bonds. Rotations were allowed along various single bonds to remove unnecessary short contacts. The molecule was also allowed to have limited translational freedom. Lowest energy position at a particular binding site was obtained. In the second step the technique of successive infinitesimal rotation (SIR) of Sklenar[38] was used to solve complicated inhomogeneous, highly nonlinear equations of energy. Translatory movements were frozen in this step. Rotations were allowed along all the single bonds shown in figures 1a,b. Change in conformation and interaction energy for rotation of the *n*th torsional angle was calculated. The next increment in the torsional angle was given proportionate to the gradient of the energy[39].

Any number of angles can be moved simultaneously in this technique. There is also a provision for changing the relative weightage for different angles. The procedure refines the geometry very fast. It has been employed efficiently in the case of DNA conformation and for drug–DNA interactions[40,41].

Energy analysis

The different contributions (attractive, repulsive, electrostatic, polarization, H-bonding and torsiona to the interaction energy for the five-membered rin and carboxy and hydroxy chains of PGs and tl polar head and aliphatic chains of DPPC we calculated.

Geometry analysis

The final geometries of the complexes, and stere chemical factors such as H-bonding geometrie distances between functional groups, etc. were als analysed.

All calculations were done on a HP 1000 A seri computer using programs specially developed for tl purpose.

RESULTS AND DISCUSSIONS

The rigid body docking procedure suggested th the polar head group of the DPPC molecule is tl main binding site for the PGs. $PGF_{2\alpha}$ could bind the polar head in two different ways. In model (figures 2a–c) 9α- and 11α-hydroxy groups a oriented towards the phosphate groups of DPP and bifurcated H-bonds are formed between O_2 ar O_3 atoms of DPPC with 9α- and 11α-hydro hydrogens. The O...H distances in this case we 2.43 Å, 1.81 Å, 3.33 Å and 2.66 Å, and the O ... H– angles 143°, 159°, 139° and 105°, all well within tl H-bonding limits (table 1). The carboxy and hydro chains of PG run parallel to the aliphatic chains DPPC on both sides (figure 2c). The five-member ring overlaps a portion of the polar head close them.

In model II the five-membered ring of PG shifted away (figures 3a, b) from the phospha group, and the 15-(S)-hydroxy group is directe towards O_2 and O_3, making bifurcated H-bon with H-bond lengths 1.85 Å and 2.23 Å and O... H– angles 155° and 98° (table 1). The hydroxy cha and the five-membered ring overlap the polar hea The carboxylic chain runs parallel to the aliphat chain of DPPC. There is a good amount of overla between the two molecules (figure 3b). Moreover, 9 and 11α, which are specific for PG activity, a oriented away from the polar head (figure 3a). Th region, as mentioned earlier[30], has the lowe electrostatic potential, compared to other PGs. It ca serve as an ideal cation-binding site.

In the case of PGB_1 the carbonyl oxygen at tl 9th position shows electrostatic repulsion with tl DPPC phosphate oxygens O_2 and O_3. Model

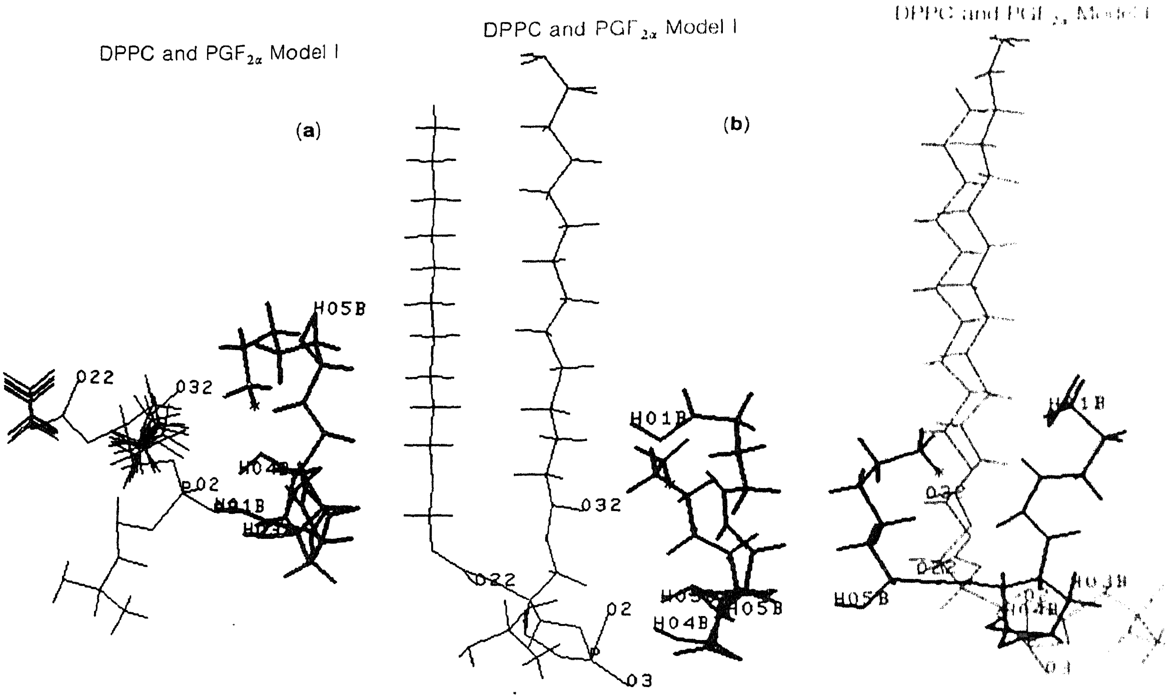
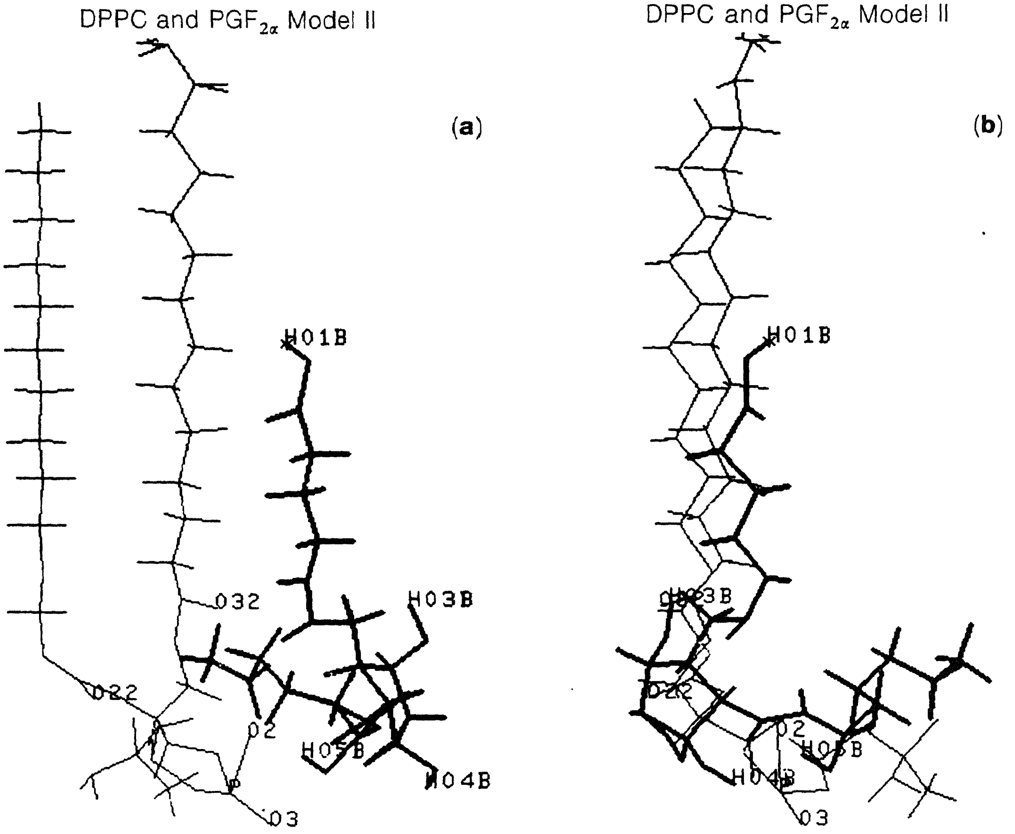

DPPC and PGF$_{2\alpha}$ Model I
(a)
DPPC and PGF$_{2\alpha}$ Model I
(b)
DPPC and PGF$_{2\alpha}$ Model I
H05B
022
032
H04B
02
H01B
H03B
032
022
02
03
H01B
H05B
H04B
H05B
H01B
032
022
H03B
H04B
03

Figure 2a–c. **a,** Computer-built model for the interaction of DPPC with PGF$_{2\alpha}$ (model I) in xy plane. Hydrogen bonding is between O_2 and O_3 of the polar head of DPPC with 11α- and 9α hydroxy groups of PGF$_{2\alpha}$. **b,** The same model in xz plane. **c,** The same model in yz plane.

Table 1 *Interatomic distances and angles between H-bonding donors and acceptors in PG DPPC models*

Model	Phosphate oxygens of DPPC	Hydroxy hydrogens of PGs	O...H distance (Å)	Angle O...H O (degrees)
PGF$_{2\alpha}$ (model I)	O_2	OH_9	2.43	143
	O_2	OH_{11}	1.81	159
	O_3	OH_9	3.33	139
	O_3	OH_{11}	2.66	105
PGF$_{2\alpha}$ (model II)	O_2	15-(S)-OH	1.85	155
	O_3	15-(S)-OH	2.23	98
PGB$_1$ (model III)	O_2	15-(S)-OH	3.42	90
	O_3	15-(S)-OH	1.96	144

could be realized only after the molecule was taken sufficiently away from the DPPC molecule (figure 4a). We attempted interaction of the 15-(S)-hydroxy group with the polar head using the crystallographic conformation of PGB$_1$[26] as the starting point. However, because the 15-(S)-hydroxy group was pointed in between the hydroxy and carboxy chains, the model was sterically not possible. We then attempted modelling this interaction starting from the conformationally optimized structure of PGB$_1$[11]. Torsional angles C_9–C_8–C_7–C_6 (α1), C_{11}–C_{12}–C_{13}–C_{14} (α9) and C_{13}–C_{14}–C_{15}–C_{16} (α10) in this case were fixed at 120°, 120° and 270° respectively. The optimized model (model II), with the 15-(S)-hydroxy group making bifurcated H-bonds with O_2 and O_3 with O–H distances 1.96 and 3.43 Å, was obtained


DPPC and $PGF_{2\alpha}$ Model II

DPPC and $PGF_{2\alpha}$ Model II

Figure 3a, b. **a**, Computer-built model for the interaction of DPPC with $PGF_{2\alpha}$ (model II), showing bifurcated hydrogen bonding between O_2 and O_3 of DPPC with 15-OH of $PGF_{2\alpha}$. In this model 9α- and 11α-hydroxy groups are free to interact with the cation. **b**, The same model in *yz* plane. The model shows stereochemical compatibility of $PGF_{2\alpha}$ with DPPC.

(figure 4b). As in the case of $PGF_{2\alpha}$, model II for PGB_1 also showed a good amount of overlap with the DPPC conformation. However, there was no specific cation-binding site in this case.

A third model (model III) could be obtained both with PGB_1 and $PGF_{2\alpha}$ with no specific H-bonding contacts with the DPPC molecule. The PG molecule in this case was deeply embedded in the aliphatic chains of DPPC (figure 4c), similar to the 'walking-stick model' of Finean[42]. The complex was stabilized in the hydrophobic region formed by the aliphatic chains of DPPC.

PG conformation

Because we allowed rotations around various single bonds (figure 1b) the conformation of PG in the optimized models was different from the crystallographic or conformationally minimized starting conformation (table 2). The conformation energy with respect to crystallographic structure could be lower and higher (table 2), because it depended on the degree of refinement in the optimized models. Comparison of the torsional angles of the final models with the crystallographic as well as energy-minimized conformations obtained in our earlier studies[25, 34] is shown in table 2. Considerable differences were observed in the torsional angles around the C_7–C_8, C_{12}–C_{13} and C_{15}–C_{16} bonds.

Inter-oxygen distances

Reduction in the distances O_9–O_{1B} and O_{11}–O_{1B} by 3 and 4 Å respectively was found in model I for

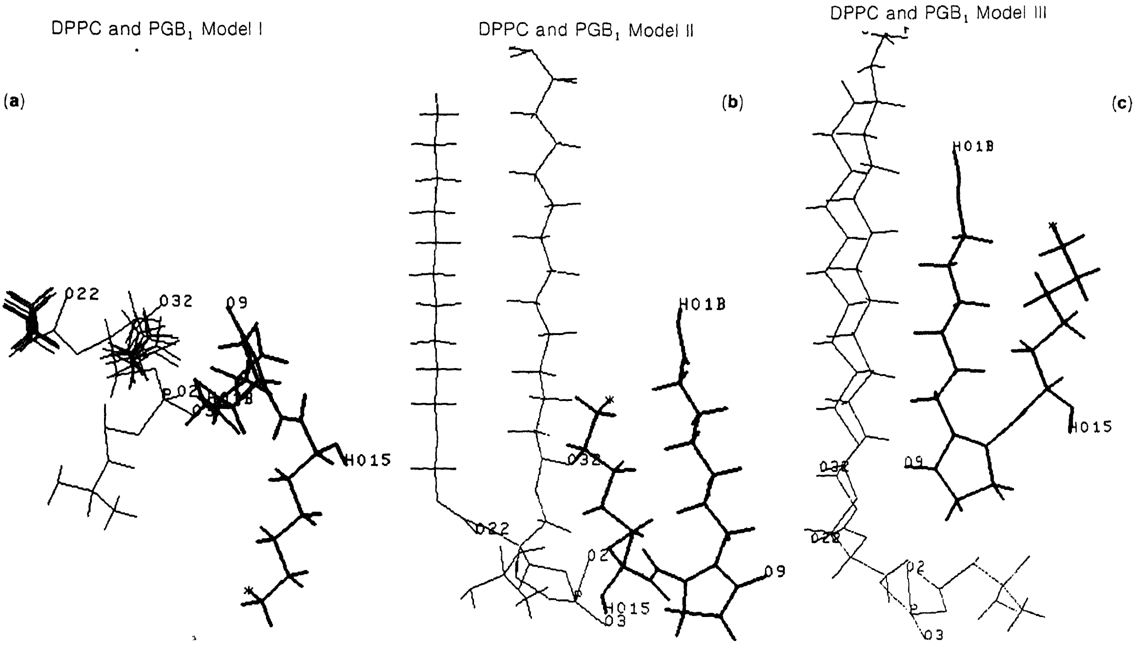


Figure 4a–c. **a,** Computer-simulated model interaction of DPPC with PGB$_1$ (model I). Here hydrogen bonding is not possible because O$_9$ of PGB$_1$ ring repels the two oxygens of the polar head of DPPC. The hydroxy chains and ring are turned away from the DPPC molecule. **b,** Computer-simulated model for the interaction between DPPC and PGB$_1$ (model II), depicting bifurcated hydrogen bonding between O$_2$ and O$_3$ of DPPC and 15-hydroxy group of PGB$_1$. Note the difference between this model and model II for PGF$_{2\alpha}$ (figure 3a, b). **c,** PGB aligned along with the aliphatic chains of DPPC (model III). The figure clearly depicts the hydrophobic interactions between the chains.

PGF$_{2\alpha}$. The O$_{1B}$–O$_{15}$ distance was reduced here by 1.72 Å. Inter-oxygen distances in model II for both PGF$_{2\alpha}$ and PGB$_1$ were of the same order as those found in the crystallographic study.

Energetics

In the case of PGF$_{2\alpha}$, model I was the most preferred model. The interaction energy in this case is lower by 12.73 kcal mol^{-1} compared to model II (table 3). Stabilization of the complex is mainly by H-bonding interaction. The dispersion contribution of 11.94 kcal mol^{-1} is compensated by electrostatic repulsion (16.6 kcal mol^{-1}) between O$_2$ and O$_3$ atoms of DPPC and ring oxygens of PG. In model II there is reduction in the electrostatic repulsion by 7 kcal mol^{-1} and increase in the dispersion by 10.5 kcal mol^{-1}. As a result both dispersion and H-bonding interactions contribute towards stabilization of the PG–DPPC complex.

The maximum contribution to the stabilization energy in model I for PGF$_{2\alpha}$ is due to interaction of the five-membered ring with the polar head of DPPC (figure 5). The aliphatic chains show a small interaction with the hydroxy chain. In model II, the maximum interaction is between the polar head of DPPC and the hydroxy chain of PGF$_{2\alpha}$. A 15% contribution to the total interaction comes from the interaction of the carboxy chain of PGF$_{2\alpha}$ with the aliphatic chains of DPPC.

In the case of PGB$_1$, model II is preferred over models I and III (table 3). It is stabilized by dispersion and H-bonding forces. There is a 5 kcal mol^{-1} electrostatic repulsion between O$_2$ and O$_3$ of DPPC and O$_{15}$ of PG. The maximum contribution to interaction energy is by the polar head of DPPC and the hydroxy chain of PGB$_1$. Interactions between the polar head and the five-membered ring, and between the aliphatic chains

Table 2 *Optimized values of the torsional angles in the different models for $PGF_{2\alpha}$ and PGB_1*

Angle	Atom–atom–atom–atom	Model I	Model II	a	b
$PGF_{2\alpha}$					
α_1	C_9–C_8–C_7–C_6	−103.88	167.47		157.39
α_2	C_8–C_7–C_6–C_5	156.07	176.57	142	167.61
α_3	C_6–C_5–C_4–C_3	185.08	167.45	153	167.36
α_4	C_5–C_4–C_3–C_2	−36.01	−176.53		182.16
α_5	C_4–C_3–C_2–C_1	96.04	−179.73		175.18
α_6	C_3–C_2–C_1–O_{2A}	107.33	−41.35		−27.29
α_7	C_2–C_1–O_1–HO_1	178.11	−177.23		177.78
α_8	C_{11}–C_{12}–C_{13}–C_{14}	84.06	−49.62		117.09
α_9	C_{13}–C_{14}–C_{15}–C_{16}	−111.56	−87.54	−150, −118	247.84
α_{10}	C_{14}–C_{15}–C_{16}–C_{17}	−29.15	−6.42		61.06
α_{11}	C_{15}–C_{16}–C_{17}–C_{18}	178.19	−177.75		178.96
α_{12}	C_{16}–C_{17}–C_{18}–C_{19}	167.65	−64.64		−178.07
α_{13}	C_{17}–C_{18}–C_{19}–C_{20}	64.54	−159.12		−180.96
α_{14}	C_{18}–C_{19}–C_{20}–C_{200}	−52.89	−66.06		−61.34

Angle	Atom-atom-atom-atom	Model I	Model II	Model III	c	d
PGB_1						
α_1	C_9–C_8–C_7–C_6	110.0	128.87	129.99	−91.9	120
α_2	C_8–C_7–C_6–C_5	165.59	167.44	170.59	170.59	
α_3	C_7–C_6–C_5–C_4	172.29	175.91	177.29	177.3	
α_4	C_6–C_5–C_4–C_3	168.49	176.33	173.49	173.5	
α_5	C_5–C_4–C_3–C_2	146.41	178.86	176.39	176.4	
α_6	C_4–C_3–C_2–C_1	−179.60	176.90	170.39	170.4	
α_7	C_3–C_2–C_1–O_{1B}	154.09	156.22	154.09	154.1	
α_8	C_2–C_1–O_1–O_{1A}	179.99	179.13	179.99	180.00	
α_9	C_{11}–C_{12}–C_{13}–C_{14}	149.98	85.77	90.01	5.0	120
α_{10}	C_{13}–C_{14}–C_{15}–C_{16}	−60.01	−76.61	−75.01	123.3	270
α_{11}	C_{14}–C_{15}–C_{16}–C_{17}	166.69	144.65	141.71	166.7	
α_{12}	C_{15}–C_{16}–C_{17}–C_{18}	179.99	−149.78	−150.01	180.0	
α_{13}	C_{16}–C_{17}–C_{18}–C_{19}	−175.19	155.18	154.81	−175.2	
α_{14}	C_{17}–C_{18}–C_{19}–C_{20}	177.59	193.09	177.59	177.6	
α_{15}	C_{18}–C_{19}–$C_{20}+d_{200}$	−59.99	−57.67	−59.99	60.0	

a, Crystallographic data from reference 20; b, Conformational data from reference 29; c, Crystallographic data from reference 21; d, Conformational data from reference 26.

Table 3 *Partitioning of interaction energy ($kcal\ mol^{-1}$) in the different models for interaction of PGs with DPPC*

		E_{non}	$E_{\text{ele+pol}}$	E_{Hyd}	E_{Int}	ΔE_{Conf}	Total
$PGF_{2\alpha}$	Model I	−11.94	16.60	−80.67	−76.01	−0.35	−75.66
$PGF_{2\alpha}$	Model II	−22.47	9.53	−50.35	−63.28	−7.27	−70.55
PGB_1	Model I	−16.47	−3.59	0.0	−20.06	+8.72	−11.34
PGB_1	Model II	−14.52	5.21	−40.25	−49.56	+9.12	−40.44
PGB_1	Model III	−23.17	0.53	0.0	−22.64	+6.19	−16.45

E_{non}, Lennard Jones 6–12 potential; $E_{\text{ele+pol}}$, Electrostatic + polarization; E_{Hyd}, H-bonding contribution calculated using 10–12 potential; E_{Int}, Total interaction energy; E_{Conf}, Difference in conformation energy with respect to crystallography.

and hydroxy chain contribute 10% of the interaction energy.

In model III, where there are no specific H-bonds, the maximum contribution to the energy comes from the interaction between the aliphatic chains of DPPC and the carboxy chain of PG. It is purely of dispersive nature. There is some interaction between the five-membered ring and the aliphatic chains,

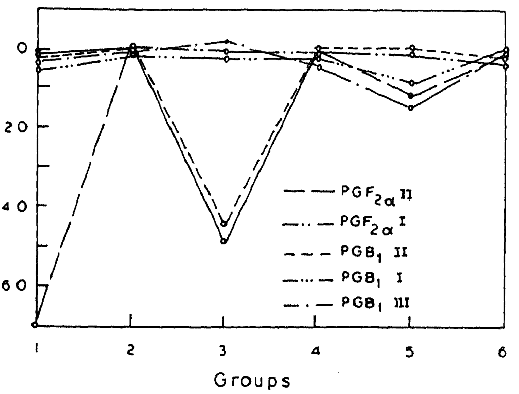


Figure 5. Partitioning of the total interaction energy for PGF$_{2\alpha}$ (models I and II) and PGB$_1$ (models I–III) with the DPPC molecule. The numbers 1 to 6 on the x-axis refer to contributions by interaction between (1) polar head of DPPC and five-membered ring of PG, (2) polar head and carboxylic chain of PG, (3) polar head and hydroxy chain of PG, (4) aliphatic chains of DPPC and five-membered ring of PG, (5) aliphatic chains and carboxy chain of PG, and (6) aliphatic chains of DPPC and hydroxy chain of PG.

which is of dispersive nature. A small amount of electrostatic interaction is found between the polar head and the hydroxy chain (figure 5).

CONCLUSIONS

The main site of interaction of PGs with membrane lipids is the polar head. The ring OH and the 15-(S)-OH groups make H-bonds with phosphate oxygens.

The conformation of PGF$_{2\alpha}$ is more suitable for interaction with the DPPC molecule. A very stable complex can be formed when 9α- and 11α-hydroxy groups of PGF$_{2\alpha}$ H-bond with O_2 and O_3 of DPPC. This interaction is stereo-specific.

It is possible that PGF$_{2\alpha}$ binds to DPPC non-specifically through the 15-hydroxy group. The 9α- and 11α-hydroxy groups in such a case point away from DPPC. Because of low-energy electrostatic potential around them, this region can serve as a good cation-binding site. Although PGB$_1$ can also bind to DPPC through the 15-(S)-OH group, the electrostatic potential around the ring is not suitable for binding to Ca^{2+}. The binding energy of PGB$_1$–DPPC is also much higher than that of PGF$_{2\alpha}$–DPPC and the former complex is energetically less favoured.

The present results show that the specificity in the molecular mechanism of action of PGs can be interpreted on the basis of their interaction with the polar head group of the lipid molecule. The orientation of the fatty acid chain of the lipid depends upon torsional angles *θ1* and *θ3* (as defined in reference 43). X-ray crystallography, NMR spectroscopy and theoretical conformation analysis[44–47] show considerable variation (from g^- to g^+) in these angles. Although these changes would modify the interaction of flexible fatty acid chains, they would have no direct influence on drug specificity since their total contribution to the stabilization energy is less than 15% (see figure 5).

To sum up, we believe that PGF$_{2\alpha}$ shows higher contractile activity because of its stereochemical compatibility with the DPPC molecule, which leads to proper orientation of functional groups. The trigger is offered by binding of PGs to DPPC through 15-(S)-hydroxy groups followed by binding of the cation to the ring hydroxy groups. PGB$_1$ is an antagonist because of the absence of this initial triggering interaction.

ACKNOWLEDGEMENT

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6 August 1988; Revised 10 November 1988

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CURRENT SCIENCE—50 YEARS AGO

ELECTRICITY IN CHEMISTRY*†

ALMOST the first application of electricity to chemistry was the experiment of Nicholson and Carlisle in which water was decomposed into its elements by a current from the voltaic pile, which had just then been discovered. This was in 1800. In the immediately following decades, the new tool of electricity held the fascination of the leading chemists. In particular, the classical work of Faraday and the laws enunciated by him have remained to this time fundamental in all considerations dealing with the relationships between electricity and chemistry.

Studies on these relationships increased rapidly, and when Arrhenius promulgated his hypothesis of ionization equilibria in solutions, a definite big impetus was given to the whole subject. The thermodynamic study of energy in chemical systems had just then been initiated and was in the full vigour of its youth. It was soon realized that the electrical energy connected with galvanic cells was of a reversible type and that rigorous methods of thermodynamics could be applied to many of the chemical changes involved in the galvanic cells. The relationships between chemical and electrical energies, particularly in liquid systems, have since been developed by systematic work into a subject in itself, under the caption of 'Electro-chemistry'. This term 'electro-chemistry' can at the present day however comprehend a much wider range of topics, for, much of the recent investigations on the electrical nature of matter and of the chemical reactions between them, the investigations mostly carried out by physicists on the electrical conductivity in solids and gases, and several other aspects of molecular physics could all be included under this head. Indeed it will be difficult to draw any sharp line between 'electro-chemistry' and other branches of physics and chemistry.

Still the old and somewhat arbitrarily defined 'electro-chemistry', which includes mostly studies of chemical reactions brought about by electrical energy and of chemical reactions giving rise to electrical energy, is itself a wide and steadily growing subject. Considerable progress has been made in this field after Debye and Huckel indicated how the then troublesome deviations of strong electrolytes from ideal behaviour in solutions, could be accounted for quantitatively by taking into consideration the interionic attractions and repulsions. As is well known the basic idea in this theory is that each ion is surrounded on the average by an oppositely charged 'ionic atmosphere' distributed with radial symmetry around the ion as centre. This concept besides its success in thermodynamics and potentiometry, has won laurels also in the field of conductometry. The ionic atmosphere gives rise to a retarding effect on the mobility of the ion, and this along with another effect pointed out by Onsager, as due to a finite time of relaxation between the ion and its atmosphere, is able to successfully account for the observed equivalent conductance of solutions of strong electrolytes at least over the range of low and moderate concentrations. Other consequences of the Debye and Huckel theory such as the Wien effect at high field strengths, and the dispersion effect at high frequencies have been predicted and verified. All these new ideas and theories have brought in their wake, considerable improvements in the technique of electrical measurement on solutions.

As regards practical applications, the principles of electro-chemistry have been utilized from the earliest days in the arts of electro-plating, electro-metallurgy, etc., while indirectly they have been of even greater use in the control of industrial processes, through conductivity, pH and EMF determinations. On the fundamental side, electro-chemistry has provided some powerful tools in investigations relating to chemical reactions involving strong electrolytes, the nature of molecular complexes, hydrolysis and solubility of substances from conductometric measurements, studies on the course of reactions from potentiometric and conductometric titrations, the analysis of proteins from electrophoretic studies as developed by A. Tiselius, etc.

**The Principles of Electrochemistry*. By Duncan A. MacInnes, (Reinhold Publishing Corporation, New York, Chapman & Hall, Ltd., London), 1939, pp. 478 with 148 illustrations, Price $ 6.00.

†Published in *Curr. Sci.*, 1939, Vol. 8, p. 330.

Among the leading contributors to this renaissance in electro-chemistry, Dr MacInnes is a prominent figure. The work of MacInnes and his associates is characterized throughout by systematic attempts to improve the accuracy of experimental data, and they have achieved many significant successes in verifying the predictions of the interionic attraction theory with respect to activity coefficients, conductivity, etc. The book itself which Dr MacInnes is now presenting to the scientific world can rightly be described as another and fundamental contribution to the science of electro-chemistry. It breathes of precision throughout: the experimental portions described deal with the modern refinements of technique while the theories are clearly and succinctly recounted by leading up from first principles wherever possible. Ample references to the original literature, most of them of recent dates, have been provided to help the reader further on. In many ways a marked and very welcome feature of the book, is the careful, painstaking and critical evaluation of the available experimental data, and of the derived physical constants. The result is, that for the field covered the book takes a front rank with other critical compilations of data on physical constants. There is, besides, a logical development of ideas, the scope of the book being extended in the last five chapters to the use of conductance measurements in various physico-chemical investigations, the effect of structure and substitution on the ionization constants of organic acids and bases, the dielectric constants of liquids and the dipole moments of molecules, electrokinetic phenomena, passivity and overvoltage.

There is but little to offer by way of helpful criticism, as it is patent that the book has been brought out with much painstaking care and with clearly defined objectives. However, a few illustrative problems either as an appendix at the end of the book, or at the end of the relevant chapters would have added to the usefulness of this book. As a work of reference to all, it is almost a necessity.

M. A. G. RAU

NEWS

METALLIC HYDROGEN (?)

Important scientific discoveries are, for some reason, first announced in the news media and only later in scientific journals! Now comes the news that at the Geophysical Laboratory, Carnegie Institution, Washington, DC, H. K. Mao, the high-pressure scientist (renowned for his remarkable work with the 'diamond anvil apparatus', which he perfected) and his collaborator Russel J. Hemley have obtained 'positive signs of hydrogen gas transforming itself into a metal'. Scientists had predicted that hydrogen may become a metal, and for almost five decades there has been a continual search for metallic hydrogen. Mao and Hemley state: 'Hydrogen was subjected to unprecedented pressures between anvils of diamond. It went through a series of transformations, and finally ended up as a black mass. This raises hopes of gaining new insights into the fundamental properties of atomic bonds.' We await the publication of the results in a scientific journal with all the experimental proofs.

VIEWS AND OPINIONS

GUIDELINES FOR INVESTIGATORS IN SCIENTIFIC RESEARCH

We publish below a document prepared by a committee of scientists of the Harvard Medical School, USA, entitled 'Guidelines for investigators in scientific research'. While not saying anything new, this document is among a few to codify the 'rules' of scientific research. Many medical researchers whom we consulted feel strongly that the document must be published in Current Science. We invite views and comments from readers on the recommendations generally and on the points made on authorship and publication practices.

—*Editor*

1. *Introduction*

These guidelines describe practices generally accepted by members of the Faculty of Medicine and already in effect in their laboratories. The primary intent of codifying them is to bring them to the attention of those beginning their careers in scientific research. These recommendations are not intended as rules, but rather as guidelines from which each group of investigators can formulate its own set of specific procedures to ensure the quality and integrity of its research.

2. *Supervision of research trainees*

Careful supervision of new investigators by their preceptors is in the best interest of the institution, the preceptor, the trainee, and the scientific community. The complexity of scientific methods, the necessity for caution in interpreting possibly ambiguous data, and the need for advanced statistical analysis, all require an active role for the preceptor in the guidance of new investigators. This is particularly true in the not uncommon circumstance of a trainee who arrives in a research unit without substantial experience in laboratory science.

Recommendations

i) The responsibility for supervision of each junior investigator should be specifically assigned to some faculty member in each research unit.

ii) The ratio of trainees to preceptors should be small enough that close interaction is possible for scientific interchange as well as oversight of the research at all stages.

iii) The preceptor should supervise the design of experiments and the processes of acquiring, recording, examining, interpreting, and storing data. (A preceptor who limits his/her role to the editing of manuscripts does not provide adequate supervision.)

iv) Collegial discussions among all preceptors and trainees constituting a research unit should be held regularly both to contribute to the scientific efforts of the members of the group and to provide informal peer review.

v) The preceptor should provide each new investigator (whether student, postdoctoral fellow, or junior faculty) with applicable governmental and institutional requirements for conduct of studies involving healthy volunteers or patients, animals, radioactive or other hazardous substances, and recombinant DNA.

3. *Data gathering, storage and retention*

A common denominator in most cases of alleged scientific misconduct has been the absence of a complete set of verifiable data. The retention of accurately recorded and retrievable results is of utmost importance for the progress of scientific inquiry. A scientist must have access to his/her original results in order to respond to questions including, but not limited to, those that may arise without any implication of impropriety. Moreover, errors may be mistaken for misconduct when the primary experimental results are unavailable. In addition, when statistical analysis is required in the interpretation of data, it should be used in the design of studies as well as in the evaluation of results.

Recommendations

i) Custody of all original primary laboratory data must be retained by the unit in which they are generated. An investigator may make copies of the primary data for his/her own use.

ii) Original experimental results should be recorded, when possible, in bound books with numbered pages. An index should be maintained to facilitate access to data.

iii) Machine print-outs should be affixed to or referenced from the laboratory notebook.

iv) Primary data should remain in the laboratory at all times and should be preserved as long as there is any reasonable need to refer to them. The chief of each research unit must decide whether to preserve such primary data for a given number of years or for the life of the unit. In no instance, however, should primary data be destroyed while investigators, colleagues, or readers of published results may raise questions answerable only by reference to such data.

4. *Authorship*

A gradual diffusion of responsibility for multi-authored or collaborative studies has led in recent years to the publication of papers for which no single author was prepared to take full responsibility. Two critical safeguards in the publication of accurate, scientific reports are the active participation of each co-author in verifying that part of a manuscript that falls within his/her speciality area and the designation of one author who is responsible for the validity of the entire manuscript.

Recommendations

i) Criteria for authorship of a manuscript should be determined and announced by each department or research unit. The committee considers the only reasonable criterion to be that the co-author has made a significant intellectual or practical contribution. The concept of 'honorary authorship' is deplorable.

ii) The first author should assure the head of each research unit or department chairperson that he/she has reviewed all the primary data on which the report is based and provide a brief description of the role of each co-author. (In multi-institutional collaborations, the senior investigator in each institution should prepare such statements.)

iii) Appended to the final draft of the manuscript should be a signed statement from each co-author indicating that he/she has reviewed and approved the manuscript to the extent possible, given individual expertise.

5. *Publication practices*

The committee has observed certain practices that make it difficult for reviewer and reader to follow a complete experimental sequence: the rapid publication of data without adequate tests of reproducibility or assessment of significance, the publication of fragments of a study, and the submission of multiple similar abstracts or manuscripts differing only slightly in content. In such circumstances, if any of the work is questioned, it is difficult to determine whether the research was done inaccurately, the methods were described imperfectly, the statistical analyses were flawed, or inappropriate conclusions were drawn. Investigators should review each proposed manuscript with these principles in mind.

Recommendations

i) The number of publications to be reviewed at times of faculty appointment or promotion should be limited in order to encourage and reward bibliographies containing fewer but more substantive publications rather than those including many insubstantial or fragmented reports. (It has been suggested, for example, that no more than 5 papers be reviewed for appointment as Assistant Professor, no more than 7 for Associate Professor, and no more than 10 for Professor.)

ii) Simultaneous submission of multiple similar abstracts or manuscripts to journals is improper.

6. *Laboratory guidelines*

Because each research unit addresses different scientific problems with different methods, each unit should develop its own specific guidelines to identify practices that seem most likely to enhance the quality of research conducted by its members. Those guidelines should be provided to the new investigator upon starting work.

SHORT COMMUNICATIONS

AXIALLY SYMMETRIC VACUUM SOLUTIONS OF THE BIMETRIC RELATIVITY THEORY

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A new theory of gravitation proposed by Rosen[1] displays the attractive features of general relativity without singularity. The bimetric theory of gravitation is based on two metric tensors g_{ij} (a Riemann tensor describing the gravitational field) and γ_{ij} (a tensor of flat space–time describing inertial forces).

The field equations of bimetric relativity are

$$K_{ij}=-8\pi kT_{ij}, \tag{1}$$

where $K_{ij}=N_{ij}-(1/2)Ng_{ij}$, and

$$N^i_j=(1/2)\gamma^{\alpha\beta}(g^{hi}g_{hj}/\alpha)/\beta, \tag{2}$$

where the (/) stands for covariant differentiation with respect to γ_{ij}, $k=(g/\gamma)^{1/2}$, $g=\det(g_{ij})$, $\gamma=\det(\gamma_{ij})$ and T_{ij} is the energy momentum tensor. In our earlier work[2,3] we observed that Rosen bimetric theory does not admit a plane-symmetric massive scalar field, an axially symmetric complex field as well as complex scalar field coupled with electromagnetic field. Moreover the Maxwell tensor vanishes in all cases contributing nothing to the gravitational field, resulting in vacuum solution. An attempt was made to derive non-static axially symmetric vacuum solutions exhibiting cylindrical waves. In this note we look for solutions of bimetric relativity with a cosmological constant, that is

$$N^i_j=\lambda\delta^i_j, \tag{3}$$

similar to the Einstein field equations $R_{ij}=\lambda g_{ij}$ of general relativity, where λ corresponds to a cosmological term. We choose the Einstein–Rosen non-static metric

$$ds^2=e^{2\alpha-2\beta}(dT^2-dR^2)-R^2e^{-2\beta}d\phi^2-e^{2\beta}dZ^2, \tag{4}$$

where α and β are functions of R and T only and $x^1=R$, $X^2=\phi$, $X^3=Z$, $X^4=T$. Now the flat space–time corresponding to (4) will be

$$d\sigma^2=dT^2-dR^2-R^2d\phi^2-dZ^2. \tag{5}$$

The non-vanishing γ-Christoffel symbols for metric (4) are

$$\Gamma^2_{12}=\Gamma^2_{21}=1/R \text{ and } \Gamma^1_{22}=-R.$$

Now the field equations in (3) yield

$$\lambda=-(\alpha''-\beta'')-(1/R)(\alpha'-\beta')+(\ddot\alpha-\ddot\beta)+(1/R^2)\sinh(2\alpha), \tag{6}$$

$$\lambda=\beta''+\beta'/R-\ddot\beta-(1/R^2)\sinh(2\alpha), \tag{7}$$

$$\lambda=-(\beta''+\beta'/R-\ddot\beta), \tag{8}$$

$$\lambda=-(\alpha''-\beta'')-(1/R)(\alpha'-\beta')+(\ddot\alpha-\ddot\beta), \tag{9}$$

where $\alpha'=\partial\alpha/\partial R$, $\dot\alpha=\partial\alpha/\partial T$, etc.

These equations imply

$$\alpha=0 \tag{10}$$

$$\beta''+\beta'/R-\ddot\beta-\lambda=0. \tag{11}$$

Case (1): Let $\beta=H(r,t)+g(t)$
then (11) gives the Bessel equation

$$H''+H'/R-\ddot H=0, \tag{12}$$

representing cylindrical waves,

provided $\ddot g=\lambda$. (13)

The Bessel equation (12) results in

$$H=aj_0(hR)\cos(ht+e_1)+bN_0(hR)\sin(ht+e_2). \tag{14}$$

From (13) we get

$g=(\lambda/2)t^2+c_1t+c_2$, where c_1 and c_2 are arbitrary constants of integration. Then

$$\beta=aj_0(hR)\cos(ht+e_1)+bN_0(hR)\sin(ht+e_2)+(\lambda/2)t^2+c_1t+c_2, \tag{16}$$

where $j_0(hR)$ and $N_0(hR)$ are the Bessel functions of the first and second kind of zero order respectively, h the frequency and a, b, e_1, e_2 are arbitrary constants.

Case (2): Let $\beta=H(r,t)+f(r)$
In this case we get (12)

provided $f''+f'/R=\lambda$. (17)

The solution of (17) is

$$f=\lambda R^2/4+c_3\log R+c_4,$$

Einstein-Rosen metric ; Bimetric Mechanics where c_3 and c_4 are arbitrary constants of integration. Now the solution turns out to be

$$\beta = aj_0(hR)\cos(ht+e_1) + bN_0(hR)\sin(ht+e_2) + \lambda R^2/4 + c_3\log R + c_4. \qquad (18)$$

For Marder's metric,

$$ds^2 = e^{2\alpha-2\beta}(dT^2 - dR^2) - R^2 e^{-2\beta} d\phi^2 - (e^{2\beta} - e^{2\gamma})dZ^2, \qquad (19)$$

the vacuum field equations yield

$$\alpha = 0$$
$$\beta'' + \beta'/R - \beta^{\cdot\cdot} = \lambda$$
$$\gamma'' + \gamma'/R - \gamma^{\cdot\cdot} = \lambda.$$

The form of γ is the same as that of β and hence we take $\gamma = n\beta$, n = constant etc. The solutions of (19) will be as in (16) and (18).

13 June 1988

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^{13}C NMR AND NEW KINETIC EVIDENCE FOR STERIC ENHANCEMENT OF RESONANCE

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AFTER the discovery[1] that there is steric enhancement of resonance in *ortho*-substituted 4-nitroanisoles and similar compounds, more evidence was found[2-11] for the phenomenon. In this communication we present further evidence for it.

The second-order rate constants for the reaction between some substituted anilines and 2,4-dinitrochlorobenzene are given in table 1. This is a reaction in which extra electron displacements accompany activation in consequence of electrical demands of the reaction centre. Thus electron-releasing groups, present *para* or *meta* to the amino group, increase the rate. The 4-methoxy group increases the rate by about ten times and the 3-methyl group by 1.3 times. When a methyl group is present *ortho* to methoxyl, the observed rate is 17.1×10^{-4}, $1\ mol^{-1} s^{-1}$, while the rate calculated on the basis of additivity of group effects[12] shows an increase of about 13%. The increased rate is due to the enhanced resonance of the methoxy group. When the methoxyl orients away from the *ortho* methyl, its rotation gets restricted and its chances of becoming coplanar with the benzene ring increase, enhancing its resonance.

Table 1 *Second-order rate constants for the reaction between substituted anilines and 2,4-dinitrochlorobenzene at 30°C*

Substituent(s)	$10^4 k_2$ ($l\ mol^{-1} s^{-1}$) Obs.	Calc.
H	1.10	–
4-Methoxy	11.4	–
3-Methyl	1.46	–
4-Methoxy-3-methyl	17.1	15.1
4-Methoxy-3,5-dimethyl	5.75	20.1

The observed rate of 4-methoxy-3,5-dimethylaniline is much less than the calculated rate, indicating the expected steric inhibition of resonance.

Proof for the existence of steric enhancement of resonance may also be seen from the ^{13}C NMR spectra of the substituted 4-nitroanisoles listed in table 2. A 2-methyl substitution in 4-nitroanisole increases the electron density at C-4, compared with 4-nitroanisole, as seen from the upfield shift and $\Delta\delta$ value, reflecting steric enhancement of resonance. For 2,6-dimethyl-4-nitroanisole there is a downfield shift, indicating decreased electron density and the expected steric inhibition of resonance.

The compounds available commercially were purified rigorously before use and others were

Table 2 *Chemical shifts of the benzene ring for substituted anisoles*

Anisole	Chemical shift of C-4 (ppm) Obs.	Calc.*	$\Delta\delta$
4-Nitro	141.5	140.8	+0.7
2-Methyl-4-nitro	140.6	140.7	−0.1
2,6-Dimethyl-4-nitro	143.2	140.6	+2.6

*Calculation is based on δ_e for benzene, 128.5, and substituent effects from ref. 15.

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prepared by standard methods described in the literature. The purity was checked by TLC. The experimental procedure followed for the kinetic study was essentially that of Singh and Peacock[13], and Reinlander[14]. The spectra were recorded on a Bruker WM-300 instrument at 75 MHz in $CDCl_3$ and referenced to TMS.

22 July 1988

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ISONITROSOTHIOCAMPHOR AS A SPECTROPHOTOMETRIC REAGENT FOR PLATINUM

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THE use of isonitrosothiocamphor (INTC) as an analytical reagent for palladium[1], cobalt[2] and copper[3] has been described earlier. At room temperature platinum (IV) shows no visible colour reaction with INTC, but forms a greenish chelate while heating on a water bath. The chelate is extractable into chloroform. A method has therefore been devised to determine platinum (IV) spectrophotometrically.

Isonitrosothiocamphor (INTC) was prepared by the reported method[4] and a solution of the reagent in ethanol (0.4%, w/v) was used. One gram of chloroplatinic acid (Johnson and Matthey) was dissolved in distilled water (100 ml) and was standardized[5]. Sample solutions of platinum were prepared by dilution of the stock solution.

Chloroform (E. Merck) was distilled before use. All other reagents used were of analytical grade. Stock solutions of the desired ions were prepared from the corresponding salts and the metal contents determined by conventional methods[5]. Potassium hydrogen phthalate sodium hydroxide buffer (pH 7–8) was used to adjust the pH of aqueous solutions. Absorbance and pH measurements were made as reported earlier[1].

To an aliquot of the sample solution of platinum (IV) (100–200 μg) was added ethanolic solution of INTC (0.5 ml of 0.4%), followed by addition of the buffer solution. The volume of the aqueous phase was maintained at 10 ml throughout the work. The mixture thus prepared was then heated (10 min) on a boiling water bath, cooled to room temperature, and then equilibrated (1 min) with chloroform (10 ml). The separated organic layer was poured over anhydrous sodium sulphate to remove any residual water. Finally the absorbance of the chloroform extract at 369 nm was measured against reagent blank. Platinum (IV) was determined from the calibration curve. To test for interference due to other ions, the ion was added to the system before addition of the reagent.

The extraction of platinum complex was investigated in the pH range 0–10. However, the chloroform extracts showed maximum and steady absorbance (for at least 24 h) when the extractions were carried out at pH in the range 6–9. The extraction of the aqueous phase was repeated till an organic extract that showed virtually no absorbance was obtained, which indicated complete and quantitative recovery of platinum from the aqueous phase.

The absorption spectrum of the Pt(IV)–INTC complex in chloroform shows absorption maxima at 369 and 435 nm. The reagent blank shows insignificant absorbances from 340 nm onwards. Hence the wavelength of 369 nm was selected for analytical measurements for higher absorbance.

It was noted that 0.5 ml of 0.4% ethanolic solution of INTC was the optimum reagent concentration to extract 100–200 μg of Pt(IV) in a single extraction. Higher reagent concentration was avoided, since in the presence of a higher concentration of INTC the absorption of the blank is high. Beer's law was obeyed over the concentration range 2–25 ppm of platinum (IV). The molar absorptivity and sensitivity index (on the basis of Pt content) of the colour system were found to be $0.82 \times 10^4 \mathrm{l\,mol^{-1}\,cm^{-1}}$ and 0.023 μg Pt/cm^2 respectively.

The precision and accuracy of the method were tested by analysing solutions containing a known amount of platinum. The average of six determinations (190 μg Pt) was found to be 191.33 μg, with a relative mean deviation of 1.91%.

A deviation of not more than ±3% from the recovery of platinum was taken as the standard tolerance limit in testing for interference due to other ions. In the estimation of 190 μg of Pt, the following ions did not interfere when present in the amounts (mg) shown in parentheses: Cd(II) (4.5), Ca(II) (5.2), Ce(III) (4), Cr(III) (4.5), Pb(II) (5.5), Mn(II) (3.2), Th(IV) (3), U(VI) (4), Zn(II) (5.5), Zr(IV) (4.5), Bi(III) (3), La(III) (4.5), Mg(II) (1), Al(III) (1), Mo(VI) (4.5). Palladium(II) (0.5), Cu(II) (0.5). These ions could be tolerated in the presence of thiosulphate and tartrate respectively. Interference due to Ni(II) (0.5) and Co(II) (0.5) was avoided by using EDTA as the masking agent. In the presence of silver(I), platinum(IV) developed no colour with INTC. High results are obtained in presence of rhodium(III).

Among the anions tested, 10 mg each of thioctanate, thiosulphate, iodide, EDTA, citrate, tartrate, oxalate and phosphate did not interfere. Less than 0.5 mg of fluoride could be tolerated. Ascorbate and thiourea interfered.

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CHEMICAL ANALYSIS OF *CALOTROPIS PROCERA* LATEX

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HESSE and his collaborators[1] detected, rather interestingly, α-lactucerol as the only constituent in the ethanolic extract of *Calotropis procera* latex. The latex also revealed the presence of a variety of cardioactive poisons, terpenoid alcohols, esters involving steam-volatile and other long-chain fatty acids[2,3], taraxasterol, calotropagenin and six of its glycosides[4–11], and three additional compounds[12]. All these glycosides contained a common aglycone[5] to which a structure[13–15] was assigned and subsequently established by its partial synthesis[16,17]. Table 1 lists the compounds hitherto identified and isolated in pure form from *Calotropis procera* latex, leaves and stem, with their molecular formulae and melting points, and the available relevant references.

Thin-layer chromatography of *Calotropis procera* latex extract revealed more than 22 spots on exposure of the plate to iodine vapour and sulphuric acid spray (60% v/v) and subsequent heating to 105°C for 5 min. This observation prompted the authors to make a systematic analysis of the latex and look for compounds yet undetected and/or unidentified.

Calotropis procera latex was collected as described earlier[18]. The dried solid was extracted exhaustively with ethanol and the pooled concentrate on filtration deposited white shining needles melting at 224–225°C. The pentacyclic triterpenol answered to the formula $C_{30}H_{50}O$ deduced from its elemental analysis and molecular weight read from its mass spectrum. The properties and melting point of the

Table 1 *Compounds isolated from Calotropis procera latex*

Compound	Molecular formula	Melting point (°C)	Reference
Calactin	$C_{29}H_{40}O_9$	265–268	12
Calotropagenin	$C_{23}H_{36}O_6$	—	12
Calotropin	$C_{29}H_{40}O_9$	181–186	i) Hesse, G., Fasola, H. and Geiger, W., *Liebigs Annln. Chem.*, 1959, **625**, 157. ii) Crout, D. H. G., Curtis, R. F., Hassall, C. H. and Jones, T. L., *J. Chem. Soc.*, 1964, 2187. iii) 12
Calotoxin	$C_{29}H_{40}O_{10}$	229–237	i) Hesse, G., Fasola, H. and Geiger, W., *Liebigs Annln. Chem.*, 1959, **625**, 157. ii) 12
α-Lactucerol	$C_{30}H_{50}O$	224–225	1,3
Procerocid	$C_{29}H_{40}O_{10}.H_2O$	222–223	12
Syriogenin	$C_{23}H_{34}O_5$	275–278	12
Taraxasterol	$C_{30}H_{50}O$	215–217	12
Uscharin	$C_{31}H_{41}NO_8S$	270–271	12
Uscharidin	$C_{29}H_{38}O_9$	298–299	12
Uzarigenin	$C_{23}H_{34}O_4$	249–250	12
Voruscharin	$C_{31}H_{43}NO_8S$	—	12
β-Amyrin	$C_{30}H_{50}O$	199–200	Present communication
α-Calotropeol	$C_{30}H_{50}O$	204–205	
β-Calotropeol	$C_{30}H_{50}O$	216–217	
3-Epimoretenol	$C_{30}H_{50}O$	223–224	
Lupeol	$C_{30}H_{50}O$	214–215	Pant and Chaturvedi, *Curr. Sci.*, 1989, **58**, 302.

compound and those of its acyl (m.p. 251–252°C) and benzoyl (m.p. 255–257°C) derivatives tallied with those of α-lactucerol and its derivatives[1]. The identity was finally established by its conversion to iso-lactucerol (m.p. 200–202°C), preparation of the latter's derivatives and comparison of their properties with those of the preparations of Hesse *et al*[1].

Curiously, however, repetition of the experiment with latex collected from different *Calotropis procera* plants from different localities in an identical manner but during different months of the year did not always yield exclusively α-lactucerol but afforded a mixture of several compounds with α-lactucerol as one of its components. This made us believe that the chemical composition of latex depends on seasonal variations, environment, soil and the maturity of the lactifer.

The saponifiable fraction of the latex obtained in the usual manner afforded a mixture of acetic, butyric and valeric acids, which were characterized[19] by TLC employing methyl acetate ammonia (95:5 v/v) as the developing solvent and alcoholic solution of methyl red as the spray reagent. The red spots appearing on an orange background, on heating the plate at 105°C for 2 min, were compared with those of reference pure known fatty acid samples.

The unsaponifiable fraction of the latex yielded a mixture of α and β calotropeols, β-amyrin, lupeol and a triterpene which showed a retention time and mass spectrum suggesting it to be 3-epimoretenol. NMR (4.6, 2H) and IR (1645 m, 980 m, 910 m) data were consistent with the presence of an isopropenyl group. The carbinol proton was 4.5, $J=6$ and $10H_3$. The NMR spectrum also revealed signals from 0.69 to 0.97 ppm (18H) and at 1.68 ppm (3H), corresponding to six angular methyl groups and one CH_3 being on a doubly bonded carbon atom respectively. The optical rotation $[\alpha]_D$ of the compound was determined to be $-2.5°$ and is in agreement with that found by Khastgir *et al*[20]. Based on the above observations the compound was confirmed as 3-epimoretenol.

Occurrence of 3-epimoretenol in *Calotropis procera* latex is being reported for the first time.

We are greatly indebted to Dr R. J. Highet, Laboratory of Chemistry, National Institutes of Health, Bethesda, USA, for the IR, NMR and mass spectra of the compounds isolated and for carrying out the GLC analysis.

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GARNET CONTENT IN THE BEACH SANDS OF GOPALPUR (BAY OF BENGAL)

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GARNET an industrially important heavy mineral, has been found to be enriched in the black sand and sand dunes of Gopalpur coast, Bay of Bengal. The average width of the beach is 45 m. The beach runs along the north–south direction and is fringed with ephemeral streams and riverlets. Dunes of various sizes occur all along the backshore region. Black sand concentrations are not uncommon on Gopalpur beach and its occurrence along the East coast of India is well known[1–6] as it is a potential source of rare-earth minerals. In the present investigation the distribution of garnet and its concentration, in relation to the prevailing hydrodynamical conditions and topography of the area along the beach, have been studied.

Sand samples were collected from three stations (Gopalpur, Arzipalleam, and mouth of Rushikulya river) along a 15 km length at 30 m intervals representing the backshore and foreshore zones of the beach (figure 1). Beach profiles were studied during the pre-monsoon (May 1987) and post-monsoon periods (December 1987). The samples were washed, dried and sieved by using ASTM sieves of 18, 60 and 230 mesh. Garnet minerals were examined using a petrological microscope (MEOPTA)[7] and the percentage number of the mineral was counted for each fraction. The size analysis of the bulk samples was made following the Folk and Ward Method[8] on $\frac{1}{2}\phi$ scale and the statistical parameters were calculated in relation to the distribution of the garnet sands.

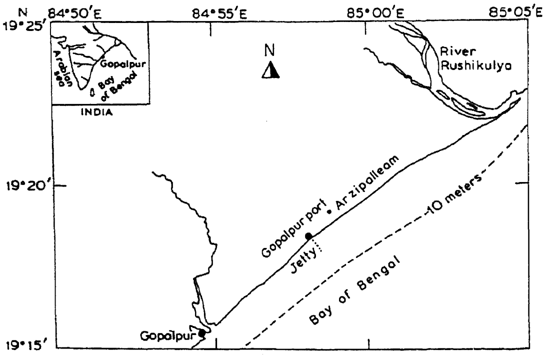

Figure 1. Location map.

Table 1 *Size parameters and garnet content of beach sands of Gopalpur*

Station	Month	Location	Mean	Sorting coefficient	Skewness	Kurtosis	Average number percentage of garnets
Gopalpur	May 1987	Backshore	0.65	0.8	−0.03	0.3	
		Foreshore	0.8	0.3	−0.07	0.4	
	December 1987	Backshore	0.62	0.6	−0.06	0.8	8.25
		Foreshore	1.05	0.4	0.09	0.7	
Arzipalleam	May 1987	Backshore	2.4	1.8	0.31	1.6	
		Foreshore	1.9	1.3	0.12	1.4	
	December 1987	Backshore	2.3	1.9	0.45	1.2	18.5
		Foreshore	1.75	1.3	0.3	0.2	
Mouth of Rushikulya river	May 1987	Backshore	2.1	1.2	0.01	0.6	
		Foreshore	1.75	1.3	0.3	0.2	
	December 1987	Backshore	1.85	1.2	0.4	0.4	12.72
		Foreshore	2.3	1.6	0.3	0.65	

Red garnet sands are abundant in the north Gopalpur coast near Arzipalleam. The size parameters and the percentage of garnet in beach sands during the period of study are presented in table 1. The garnet content in dune sands of the backshore zone varies from 8 to 48% in the coarser fraction. In the foreshore zone it varies from 20 to 30% in finer fractions and from 8 to 43% in the coarser fraction. The garnet percentage increases from the mouth of river Rushikulya to Arzipalleam and decreases to the south of Gopalpur. The maximum value was noted during the pre-monsoon and the minimum during the post-monsoon period. Fine garnet sands in river-borne sediments are deposited on the beach at the offset of monsoon and swell wave conditions in the sea.

Beach profile measurements and sediment budget study[9, 10] further reveal that the area is affected by alluvial sands. The beach in the north is very flat. The flatness favours progradation and deposition of black sands. The beach at Gopalpur was composed of very well sorted sands throughout the period of study and showed no monsoonal variation. This may be because the presence of the temporary jetties at 3 km to the north of Gopalpur might be obstructing sediment flow further southward during monsoon periods.

30 September 1988; Revised 10 November 1988

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A REPORT ON THE OCCURRENCE OF MICROVERTEBRATES IN THE SUBATHU FORMATION (MONTIAN–EARLY LUTETIAN) NEAR NILKANTH, GARHWAL HIMALAYA, UTTAR PRADESH, INDIA

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A reconnaissance survey of several sections of the Subathu Formation near Nilkanth in the Garhwal

Himalaya has recently provided many microvertebrate-producing localities in the marine sequence of the lower and middle parts of this formation. Although occurrences of fragmentary fossil bones in Garhwal were reported as early as in 1887, there was no detailed account of vertebrates from the Subathu Formation of Uttar Pradesh. In 1887 Middlemiss[1], while describing the geology of Garhwal Himalaya (then referred to as West British Garhwal), had briefly mentioned the occurrence of fragmentary bones in Subathu Formation exposed in the Tal Valley. In the subsequent one hundred years, no vertebrate palaeontological studies were undertaken in this area.

The vertebrates reported herein were obtained from four localities—two around Nilkanth, one in the Tal Valley, and one near Dogadda. Near Nilkanth microvertebrates were discovered from two sections—the first one is exposed on the Luxmanjhula–Deoli Road about 4 km short of Deoli, and the second one lies on the Deoli–Nilkanth bridal path. Both sections have yielded microfish remains (mainly ray fish) in the greyish-black recrystallized limestone that occurs as thin bands sandwiched between greyish-green shales of the middle portion of the Subathu Formation. The greyish-black recrystallized limestone is also the main microfish-yielding lithology in the Type area of Subathu Formation[2].

In the Tal Valley, the bone-bearing dark grey shale (silty at places) is exposed near the origin of a bridal path that leads to the village Talla Banas. From this locality only fragments of isolated long bones and some turtle bones were recovered. Near Dogadda the fossiliferous section is exposed on the Dogadda–Kotdwar Road near a kilometre stone showing Kotdwar 13 km and Dogadda 2 km. Here a thin bed (< 5 cm) of pebbly sandstone, consisting of iron oxide pebbles and quartz grains, yields vertebrate fossils. This horizon constitutes the basal part of the Subathu Formation and occurs intercalated with black carbonaceous shales that are rich in small bivalves and echinoid spines.

The present collection consists mainly of microfish remains that include rays and sharks. The former are represented by isolated teeth of male and female individuals of *Subathunura casieri* Kumar and Loyal, 1987, and *Dasyatis rafinesquei* Kumar and Loyal, 1987, and the latter are known by fragmentary teeth probably belonging to the family Carcharhinidae. The ray taxa *S. casieri* and *D. rafinesquei* reported in this paper have also been recently described from the Subathu Formation of Type Area[2], hence only brief descriptions are given here.

Subathunura casieri Kumar and Loyal, 1987

Crown of tooth of a male individual has a more or less vertically placed wide and subrectangular labial apron extended lingually in the form of a cusp; root robust, bilobate, wider than the crown; root lobes squarish and subrounded with a short and wide furrow in between (figure 1).

Teeth of female individuals very transverse, crown elliptical to subovate and anteroposteriorly compressed; lateral angles of crown usually sharp, occlusal surface in most cases with a transverse furrow along its width (longer diameter), margins of furrow irregular (crenulated) or entire; root massive, bilobate, wider than the crown, root lobes subovate to subrounded (figures 2–3).

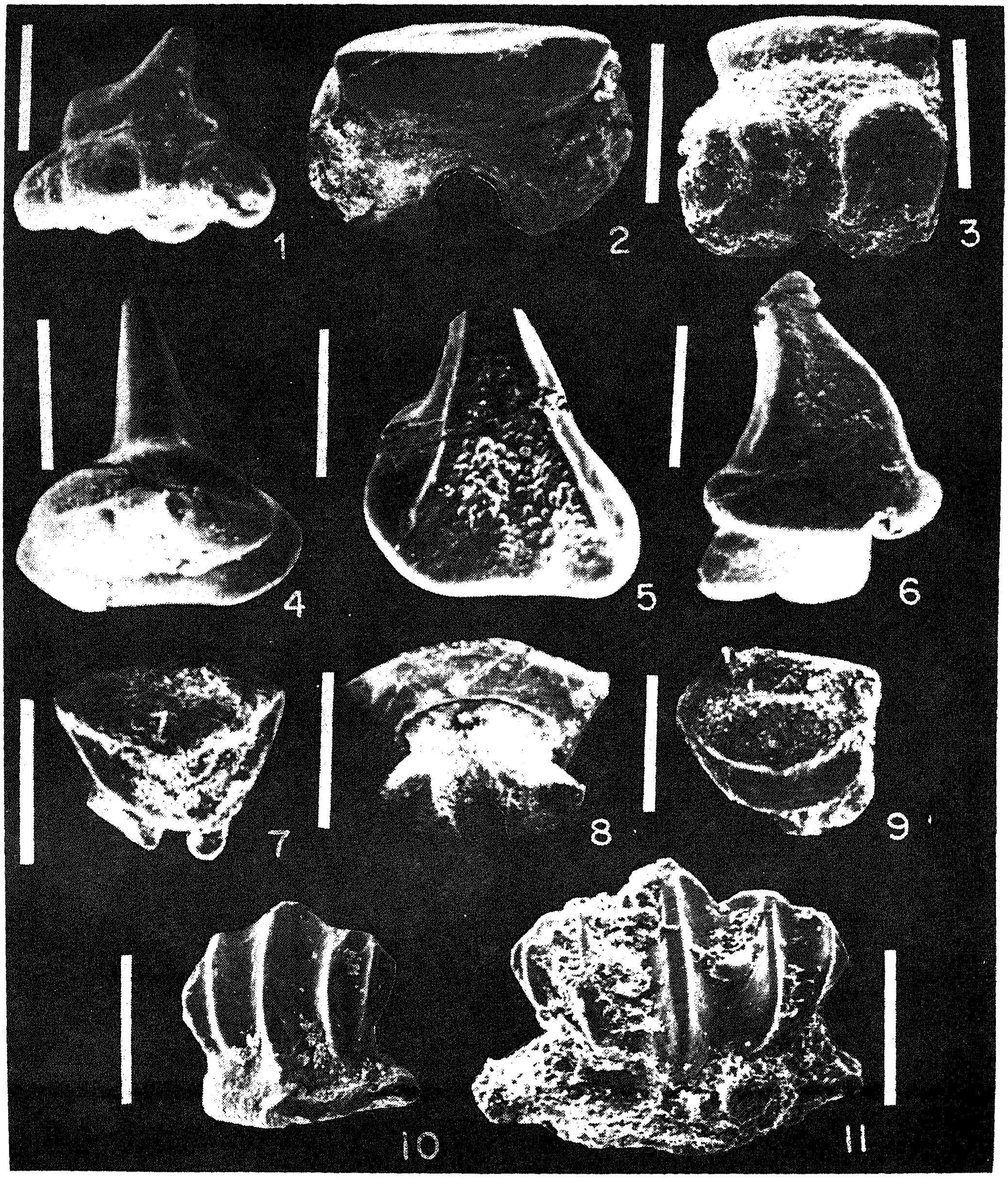
Dasyatis rafinesquei Kumar and Loyal, 1987

Teeth of male individuals with a long inclined cusp; in most teeth labial portion of crown subelliptical with more or less acute lateral angles (e.g. in WIF/A 507), in others subovate with one lateral side usually blunt (e.g. in WIF/A 510); occlusal surface of crown finely pitted, lateral sides and ventral surface smooth; root reduced with two subtriangular to subrounded lobes and a short furrow bearing a prominent nerve opening (figures 4–6).

Teeth of female individuals with low noncuspidate crown; anterior face of crown flat and elliptical to subelliptical ornamented uniformly with fine, rounded pits, posterior face smooth and slightly convex with a shallow lip, basal face more or less flat and subtriangular; root canal fairly wide with a distinct nerve opening (figures 7–9).

Associated with dental elements of sharks and rays were found many cuspidate-type dermal denticles or placoid scales and some highly pyritized ostracodes from Luxmanjhula–Deoli road section. The pyritized ostracodes are already known from Nilkanth area and the patterns of pyritization have been studied earlier[3].

The present assemblage of vertebrate fossils is strikingly similar to that from the Type Area of the Subathu Formation in Himachal Pradesh, indicating a more or less continuous basin of deposition. In the Type Area, however, the assemblage known at present is much more diversified and rich, but that



Figures 1–11. **1–3,** *Subathunura casieri*, isolated teeth of male (**1,** WIF/A 501) and female (**2,** WIF/A 503; **3,** WIF/A 504) individuals in lingual, occlusal and lingual views respectively. **4–6,** *Dasyatis rafinesquei*, isolated teeth of male individuals in linguobasal (**4,** WIF/A 508), labial (**5,** WIF/A 510) and laterolabial (**6,** WIF/A 507) views. **7–9,** *D. rafinesquei*, isolated teeth of female individuals in labial (**7,** WIF/A 514), lingual (**8,** WIF/A 513) and labial (**9,** WIF/A 512) views. **10** (WIF/A 505) and **11** (WIF/A 506), Selachii indet., cuspidate-type placoid scales in front view. (Bar in figures 1–9 represents 0.5 mm; bar in figures 10 and 11 represents 0.2 mm).

could be because of the fact that Type Subathu sediments have been explored for a longer period and far more extensively than the Uttar Pradesh Subathu, which so far have only been test-sampled. More frequent oscillations in the water level and a shallower sea, as evident from the presence of red shale bands throughout the Subathu sequence in the Garhwal Himalaya, could be other reasons for the less diverse and deficient fish assemblage. This situation is different from the Type Subathu and Kalakot localities where the red beds occur mainly in the topmost portion of the formation comprising transitional (= passage beds) sequence. In these localities the transitional red beds yield diverse terrestrial mammalian fauna of Lutetian age, but in Nilkanth and Dogadda none of the red shale bands have so far been found to embody vertebrates. This could probably be due to two reasons: first, these red beds are not transitional, and secondly, they are older than those of Kalakot and Subathu areas and are of Ypresian age. Invertebrate palaeontological evidences also support a Montian to Early Lutetian age range for the Subathu Formation of Garhwal Himalaya[4].

24 November 1988

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ECHINOCATENA ARTHRINIOIDES CAMPBELL—A NEW GENERIC RECORD FOR INDIA

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DURING 1980 a rare and interesting hyphomycetous fungus of the family Dematiaceae and identified as *Echinocatena* was isolated from decaying teak leaf litter of dry deciduous forest of Jabalpur, Madhya Pradesh. The genus *Echinocatena* was erected by Campbell and Sutton in 1977 with the type species *E. arthrinioides*[1]. According to them, this genus shows close relation with *Trichobotrys* Sacc., *Sadasivania* Subram. and *Parapericonia* Ellis *et al*[3,4]. A perusal of the literature reveals that this genus has so far not been reported from India[4].

On decaying leaves minute 1-mm diameter colonies were observed during September to November. The colonies were first light brown, then changed to dark brown to black, and were irregular in outline. Mycelium immersed and superficial, formed of branched, septate, pale brown, smooth hyphae 2–2.5 μm wide; conidiophores formed from the superficial mycelium, 10–23 × 2–2.5 μm broad, micronematous, mononematous, unbranched, straight, pale brown, sparsely echinulate or smooth; conidiogenous cells arising in simple or branched acropetal chains from the conidiophore apex, 5–10.5 × 3.5–5 μm, separated by prominent, thick, dark brown septa, pale brown, echinulate, cylindrical to doliform, constricted at the septa, polyblastic, integrated, indeterminate; conidia 3.5–4.5 μm diameter, solitary, dry, spherical, brown, thick-walled, aseptate, echinulate, appearing as vertical cylindrical columns up to 35 μm in length (figure 1).

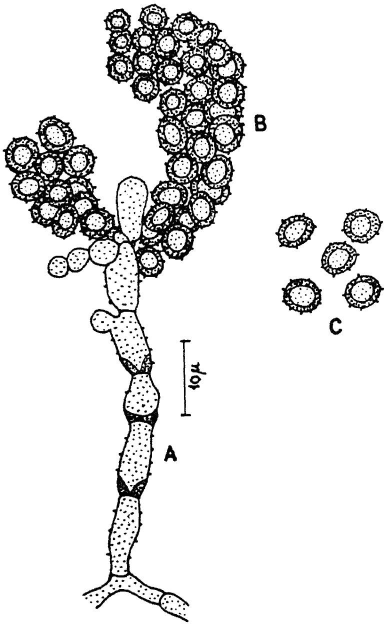

Figure 1. *Echinocatena arthrinioides* Campbell. **A**, Conidiophore; **B**, column of conidia; **C**, conidia.

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*For correspondence.

Growth on PDA and MA was very slow. Immersed mycelium composed of dark brown septate hyphae; aerial mycelium abundant, denser and more compact towards the centre, becoming floccose, sparse and finally absent from the advancing edge; reverse brown, vinaceous; sporulation abundant. The culture of *Echinocatena arthrinioides* was also tested for cellulase production but it was unable to produce C_1 or C_x activities on cellulosic substrates.

The fungus was isolated from decaying leaf litter of *Tectona grandis* (Verbenaceae), Pariyat Forest, Jabalpur. Dec. 1980, IMI No. 254970.

The authors are grateful to the Director, Commonwealth Mycological Institute, Kew, England, for his help in identification of the fungus.

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A NEW LEAF SPOT ON *TRICHOSANTHES ANGUINA* L. CAUSED BY *PHAEOTRICHOCONIS CROTALARIAE* (SALAM & RAO) SUBRAM.

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A new leaf spot disease was seen on *Trichosanthes anguina* L. (snake gourd) in a 30-day-old crop at this Institute, in March 1987. Both young and old leaves were affected. The spots were irregular, measuring 1–4 mm in length and 1–2.5 mm in breadth, and pale brown in colour. They were seen on the lower surface of the leaves on the interveinal space; the corresponding regions of the upper surface turn pale brown. Numerous spots were seen scattered all over the leaf surface. When incubated on oat agar, a mycelium developed.

Mycelium light brown, septate 3.5 to 6.0 μm thick, branched; multiseptate conidia, borne singly at the tip of conidiophores, after 15 days of incubation at 35 ± 2°C; conidiophore hyaline or brown in colour and not sharply distinguishable from the hyphae; conidia brown to dark brown, acrogenous, 4- to 6-septate, not constricted at the septa, with a definite beak measuring 58.5 × 25.7 μm (42–78 × 12–18 μm); a definite scar at the base of the conidium at the point of attachment with the conidiophore; end cells hyaline and middle cells brown to dark brown; second and third cells larger than the rest; sclerotia seen in plenty in culture after 15 days, irregular in shape, dark brown, 0.5 to 3.0 μm in size.

The isolate was pathogenic to *T. anguina* when inoculated. The fungus was identified as *Phaeotrichoconis crotalariae* (Salam & Rao) Subram. by Dr Sutton and Dr P. M. Kirk, International Mycological Institute, Kew, England, under IMI Number 317151 & 317152. This is the first report of this fungus on *T. anguina* L.

The authors thank Dr Sutton and Dr Kirk, International Mycological Institute, Kew, England, for identifying the fungus.

12 September 1988; Revised 22 December 1988

PYTHIUM INTERMEDIUM CAUSING ROOT ROT OF *OROXYLUM INDICUM* IN KERALA—A NEW INDIAN RECORD

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WHILE raising nurseries of different moist deciduous tree species for afforestation work, a severe root rot disease of seedlings of *Oroxylum indicum* Vent, a medicinal plant of the family Bignoniaceae, was recorded during October–December 1987. The disease appeared in the form of irregular patches in seed-beds and caused slow wilting of seedlings. The first symptom was the change of pigmentation of a few leaves from normal green to yellow. Subsequently all leaves were affected, and the apical portion of the seedlings wilted. The root system (feeder roots as well as main roots of such wilted plants) was found to be completely rotten (figure 1). The colour of the affected tap root was light brown to dark brown

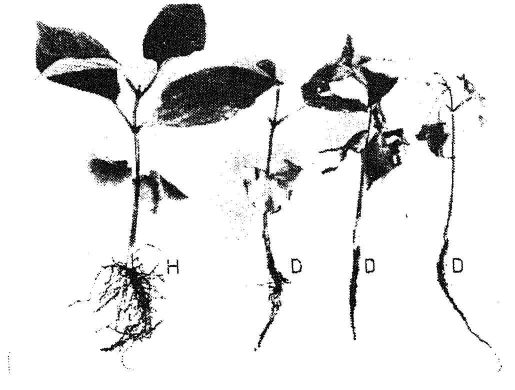

Figure 1. H, Healthy seedling of *O. indicum*; D, root rot diseased seedlings showing various stages of infection.

while roots of healthy plants were white. In some affected specimens even the root collar zone was affected, causing decay. Consistent isolation from the infected roots yielded *Pythium intermedium* de Bary (IMI No. 316590) on potato dextrose agar medium (PDA).

The pathogenicity of the isolate was tested on 2-month-old seedlings raised in pasteurized soil. For inoculation, cultures were raised on corn meal sand medium, blended and mixed separately with sterile soil in aluminium trays, and incubated for 5–7 days. *O. indicum* seedlings were transplanted after washing the root system thoroughly with sterile water. All the trays were transferred to a humidity chamber with >95% RH, and the temperature between 25 and 31°C. Observations on the appearance of disease symptoms were recorded every day.

Typical symptoms of root rot developed after 5–7 days of incubation. On the tenth day most of the seedlings died owing to severe rotting of roots. The pathogen was re-isolated from the infected seedlings.

Pythium intermedium de Bary, a typical soil-borne pathogen, has a world-wide distribution, but predominates in the temperate zone. In temperate countries, *P. intermedium* is known to cause damping off and root rot in seedlings of ornamentals[1,2] and forest tree species[3,4].

Earlier *Synchytrium cassiae*, *S. oroxyli* and *Cercospora oroxyli*[5-7] were reported from India on *O. indicum*. This is the first report from India of *P. intermedium* causing root rot of *O. indicum*.

The authors thank Dr D. J. Stamps, CAB International Mycological Institute, England, for confirming the identity of the fungus.

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RECORD OF *MIMELA XANTHORRHINA* HOPE (COLEOPTERA: SCARABAEIDAE), AS A NEW PEST OF CARDAMOM *ELETTARIA CARDAMOMUM* MATON

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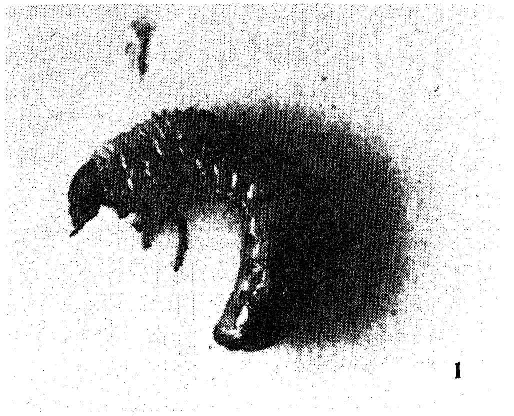
WHILE surveying cardamom plantations for insect pest infestation during February 1988, grubs of *Mimela xanthorrhina* Hope were found to cause severe damage to the roots of cardamom at Anemahal, Sakleshpur (12°56′30″ N, 75°48′38″ E, 976 m AMSL) in Hassan district of Karnataka.

The grubs were curved, black, flat-bodied, with brown head and fleshy abdominal ends (figure 1). They were found below the cardamom rhizome in soil, 4–6 inches deep. On an average, 4 grubs/clump ($n = 50$ clumps) were found feeding on cardamom roots. The rhizome portion remained undamaged. When grubs were offered roots of two-year-old cardamom plants in the laboratory in a cage (72 cm^3), the grubs cut the roots vertically (figure 3). In the field, severely affected cardamom clumps showed stunted growth and the roots lacked matty structure. No new suckers and roots were noticed in affected clumps. The existing suckers showed yellowing and wilting symptoms.

The grubs were collected and enclosed in glass jars (36 × 15 cm) with soil. The beetles emerged in the glass jar in May. The beetles (1.6 × 1.1 cm) were shiny, olive green on dorsal side and coppery brown ventrally (figure 2). When the same cardamom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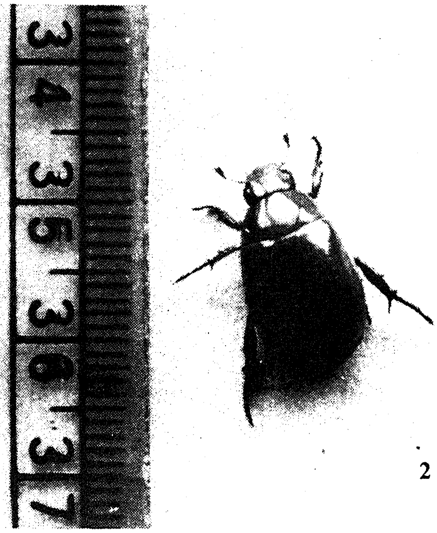
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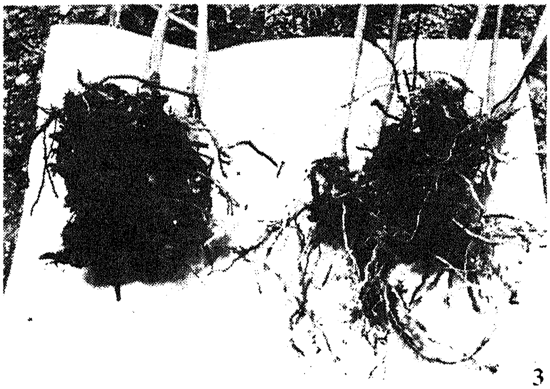
Figures 1 and 2. *Mimela xanthorrhina.* **1**, Grub; **2**, Adult.

plantation, extending over a hectare, was visited in June after the rains, a large number of the beetles were observed on the shade tree, *Elaecarpus tuburculatus* Roxby. When fresh twigs of *E. tuburculatus* were offered to beetles in the laboratory, the beetles fed voraciously on the leaves. The observations confirmed that *M. xanthorrhina* grubs were implicated in damage to roots of cardamom and the adults in damage to foliage of *E. tuburculatus* in Sakleshpur. *M. xanthorrhina* has earlier been reported only on tea[1].

Of the 208 shade trees in one acre of the cardamom plantation sampled, *E. tuburculatus* represented 18.27% and all the trees were severely defoliated by adult *M. xanthorrhina*. Almost every twig in all the plants was damaged by the beetles (figure 4). Three to four mating pairs per branch of *E. tuburculatus* could be noticed on the trees. This suggests that *Mimela* adults prefer *E. tuburculatus* for feeding and mating.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Figures 3 and 4. **3**, *Mimela*-damaged roots (left); undamaged roots (right). **4**, Twigs of *E. tuburculatus* damaged by adult *Mimela*.

Table 1 *Extent of damage caused by grub of Mimela xanthorrhina to cardamom roots*

Sample no.	Plants	Per cent damage
1	18	83.3
2	15	80.0
3	21	80.9
4	30	70.0
5	36	63.3
6	43	20.93
7	41	70.0
8	52	69.23
9	61	44.26
10	59	71.18
Total	376	

An assessment of the extent of the damage to cardamom was made by random sampling. The insect affected 58.70% of cardamom clumps (table 1).

This is the first record of *M. xanthorrhina* feeding on cardamom roots. Further studies on the insect are in progress.

Thanks are due to Dr R. B. Madge of CAB International Institute of Entomology, London, for identification of the insect.

6 September 1988; Revised 2 November 1988

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ROOTING OF EXCISED LEAVES OF GRAIN LEGUME CROPS

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AS reported previously[1] excised leaves of mungbean cultured in water developed roots without any application of plant growth regulators (PGRs). They could be maintained for up to 40 days. This simple technique has been found useful in screening for powdery mildew reaction under controlled environment and is now routinely used in our laboratory. The usefulness of this technique for screening for other foliar diseases is being assessed. This communication reports similar rooting in cultured excised leaves of other grain legume crops, particularly in pigeon pea (*Cajanus cajan* (L.) Millsp.), soybean (*Glycine max* (L.) Merr), chick pea (*Cicer arietinum* L.) and groundnut (*Arachis hypogaea* L.). Rooting in excised leaves of groundnut and soybean has been reported previously[2,3]. In soybean PGRs were used for rooting. However, PGRs are reported to alter disease reaction of barley leaves[4] and it would be desirable to avoid their use in experiments aimed at studying host–pathogen interactions.

Leaves from field-grown plants of pigeon pea, chick pea, soybean, groundnut and black gram cultivars were used (table 1). The third leaf from the base of 25-day-old seedlings was excised above the pulvinus and inserted through a series of holes in opaque plastic sheets and held in position with cotton plugs. These plastic sheets with leaves were placed over enamel trays containing tap-water so that the lower 2 cm of the petiole was always immersed[1]. The trays were kept in a growth chamber maintained at 21°C, 24°C or 28 ± 1°C, with a 12 h/day illumination of 4136 lux.

Table 1 *Cultivars of the different grain legume crops tested for rooting in excised leaves*

Crop	Cultivars
Soybean	Monetta, PK-472, CO-1, Macs-13
Chick pea	Chaffa
Groundnut	JL-24, TG-17, TG-9, SB-11, Robut
Black gram	TAU-1, T-9, LBG-17
Pigeon pea	TT-5, TT-6, TAT-10

At 21°C root initiation was observed in soybean and chick pea after 9–12 days and in groundnut and pigeon pea after 15–18 days (figure 1). The rooted

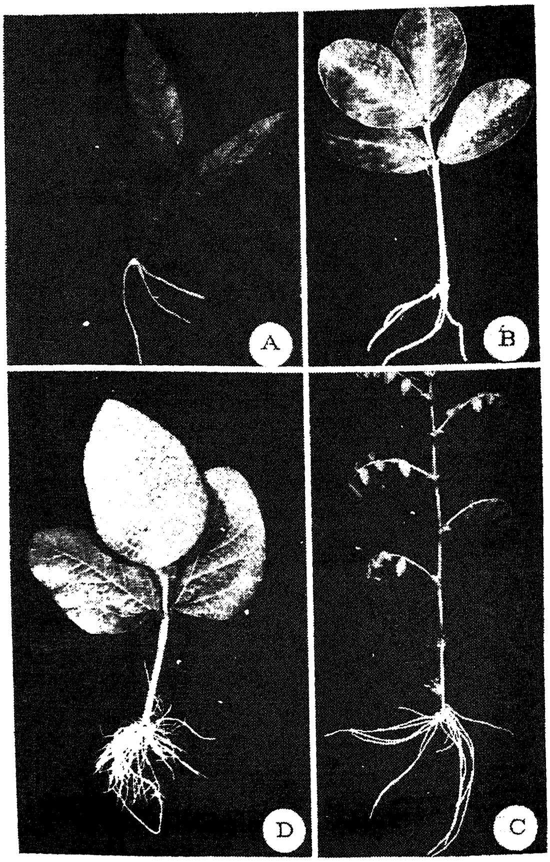

Figure 1. Excised leaves of pigeon pea (A), groundnut (B), chick pea (C), and soybean (D), showing profuse rooting from the basal region of petioles.

Table 2 *Excised leaf culture of different grain legume crops at 21 ± 1°C*

Crop	No of leaves cultured	Rooting and survival up to 40 days
Soybean	140	140
Chick pea	140	140
Groundnut	140	140
Black gram	140	136*
Pigeon pea	140	134

*Rooting was not observed; excised leaf survival up to 30 days.

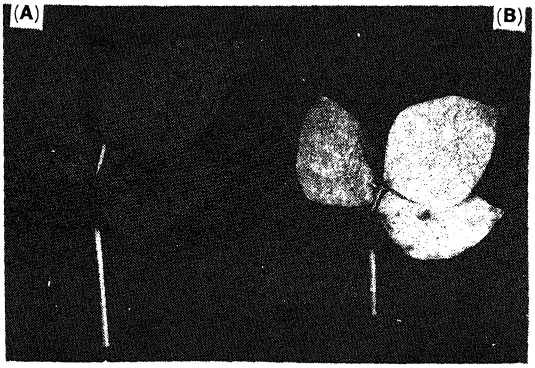

Figure 2. Resistant (LBG-17) (A) and susceptible (T-9) (B) reaction to *Erysiphe polygoni* DC. on excised leaves of *Vigna mungo* (L.) Hepper 16 days after inoculation; distinct disease lesions are seen in the susceptible type (B).

leaves could be maintained for up to 40 days, with 100% survival in soybean, chick pea and groundnut and 96% in pigeon pea (table 2). Though there was no rooting in black gram, 97% of leaves could be maintained for up to 30 days. This period was found adequate for screening for powdery mildew reaction (figure 2). At 24°C all the excised leaves survived in groundnut and chick pea, but survival was 85%, 89% and 90% in pigeon pea, soybean and black gram respectively. At 28°C survival of excised leaves of chick pea, groundnut, soybean, pigeon pea and black gram was reduced to 44%, 31%, 19%, 12% and 8% respectively. The study shows that cultured excised leaves would be ideal for evaluation of plants for foliar disease reactions under controlled environment.

3 November 1988

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GROWTH ANALYSIS IN SIRATRO AS INFLUENCED BY GIBBERELLIC ACID AND BORON TREATMENTS

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GROWTH analysis is a technique that separates growth into component processes to reveal the effects of endogenous and exogenous influences, focusing on rates of growth rather than on the final yields. The present investigation was aimed at assessing the effects of gibberellic acid (GA) and boron on growth parameters of Siratro (*Macroptilium atropurpureum*).

Healthy seeds of Siratro were sown in pots filled with soil and 45-day-old plants were sprayed with aqueous solutions of GA (25, 50 and 100 ppm) and boron (0.5, 1 and 2 ppm). A set of untreated plants served as control. Various growth parameters were calculated from fresh weight and dry weight. Leaf area data were also obtained for successive stages of plant growth[1].

Crop growth rate (CGR), relative growth rate (RGR) and net assimilation rate (NAR) were influenced by GA and boron treatments (figure 1). CGR and RGR were maximum at 50 ppm GA but decreased at the highest concentration (100 ppm) of GA. In the case of boron-treated plants, 1 ppm produced increase in CGR and RGR. These growth rates attained maximum in the earlier stage (15–30 days) of plant growth and subsequently decreased. NAR was also increased at lower doses of GA and boron, but the highest dose had inhibitory effect. In general, NAR increased in early stages of plant growth but decreased at senescence. GA increases plant growth and total dry weight resulting in increased dry matter accumulation in different plant organs[2]. Leaf area ratio (LAR) decreased with age. In the GA-treated plants, LAR increased with increasing dose of GA[3]. In the case of boron, 1 ppm gave the highest LAR among the three doses.

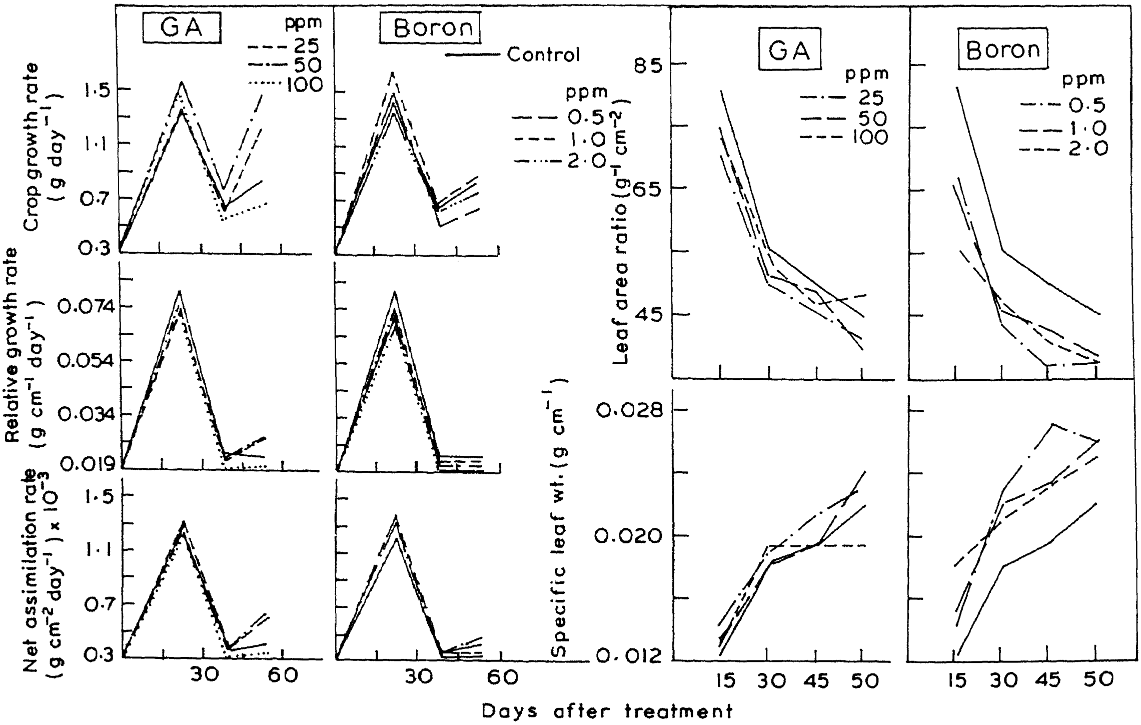

Figure 1. Effect of GA and boron on growth parameters of Siratro.

Specific leaf weight (SLW) increased with age, and was maximum at 50 ppm GA and 1 ppm boron. SLW has been shown to be related to NAR activity[4]. The present results are also supported by the findings of Dong and Arteca[5] on tomato plants treated with phytohormones.

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RESPONSE OF DIFFERENT RICE CULTIVARS TO *AZOSPIRILLUM* INOCULATION

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AZOSPIRILLUM is known to fix atmospheric nitrogen and increase the yield of several crops such as rice, wheat, maize, sorghum and pearl millet[1,2]. *Azospirillum* is a microaerophilic bacterium and survives well and fixes nitrogen in rice rhizosphere[3]. The bacterium promotes plant growth also by mechanisms other than nitrogen fixation in rice[4,5]. It produces auxins in culture and in its natural habitat[2,6]. Differential varietal response to *Azospirillum* has been reported in wheat and sorghum[7]. Hence we assessed the responses of different rice varieties to *Azospirillum lipoferum* Tarrand *et al.*

Peat-based *Azospirillum* inoculum containing approximately 10^8 cells/g was used. Inoculation was by three methods. Seeds were first treated with 2 kg of inoculum by soaking the seeds in water containing the inoculum for 24 h. For 60 kg of seed required for

Varietal response

Table 1 *Effect of Azospirillum inoculation on straw and grain yield of different rice cultivars*

Cultivar	Straw yield (t/ha)			Grain yield (t/ha)		
	Without *Azospirillum*	With *Azospirillum*	Per cent increase	Without *Azospirillum*	With *Azospirillum*	Per cent increase
Dry-season						
ADT 36	4.7 ± 0.2	5.9 ± 0.5	25.5	4.0 ± 0.1	5.0 ± 0.2	25.0
ADT 37	4.6 ± 0.3	5.8 ± 0.2	26.1	3.9 ± 0.2	4.8 ± 0.2	23.1
IR 50	4.2 ± 0.3	5.7 ± 0.7	35.7	3.6 ± 0.5	5.0 ± 0.4	38.8
TKM 9	5.4 ± 1.0	6.9 ± 1.3	27.7	4.4 ± 0.7	5.4 ± 0.9	22.7
CD ($P = 0.05$)		1.1			0.9	
Wet-season						
ADT 38	4.8 ± 0.2	5.7 ± 0.7	18.8	4.2 ± 0.1	5.0 ± 0.3	19.0
Co 43	5.3 ± 0.3	6.8 ± 0.4	28.3	5.1 ± 0.3	6.1 ± 0.1	19.6
IR 20	6.2 ± 0.8	7.3 ± 0.4	17.7	5.5 ± 0.3	6.6 ± 0.3	20.0
White Ponni	6.5 ± 0.4	8.4 ± 1.0	29.2	4.9 ± 0.7	5.8 ± 0.8	18.4
CD ($P = 0.05$)		0.9			0.8	

All values are mean ± SD.

one hectare, 60 l of water were used for soaking the seeds. The soaked seeds were allowed to sprout for 24 h and sown in the nursery. At the time of transplantation, the roots of 25-day-old seedlings were dipped in 400 l of water containing 2 kg of inoculum for 20 min. Another 2 kg of inoculum was mixed with 15 kg sand and broadcast in the main field just before transplanting[8].

The trials were conducted in both dry and wet seasons of 1987–88. During the dry season, short-duration (105–110 days) varieties, viz. ADT 36, ADT 37, IR 50 and TKM 9, were tested. During the wet season, medium-duration (130–135 days) varieties, viz. ADT 38, Co 43, IR 20 and White Ponni, were tested. The soil was fertilized with 75 kg nitrogen, 37.5 kg phosphorus and 37.5 kg potassium for dry-season crops and 75 kg nitrogen, 50 kg phosphorus and 50 kg potassium for wet-season crops. While both phosphorus and potassium were applied basally, nitrogen in the form of urea was applied in three split doses (50% at planting, 25% at 15 days after transplanting and 25% at panicle initiation) for dry-season crops and in four splits (28% at planting, 24% at 15 days after transplanting, 24% at maximum tillering and 24% at panicle initiation) for wet-season crops. Plant height and number of productive tillers/hill were measured on day 100 after sowing. Twenty-five plants selected at random were assessed for plant height and for number of productive tillers. Straw (dry) weight of the plants and grain yield at 12% moisture were also recorded. Three replicates were employed and the data were statistically analysed.

Azospirillum application increased plant height in all the varieties tested. There was also an increase in the number of productive tillers due to bacterial inoculation in all the cultivars. Increased growth rate of rice plants due to *Azospirillum* has been reported[5,9]. This increase may not be due to nitrogen fixation by the bacterium alone, as clearly demonstrated by Watanabe and Lin[5], using ^{15}N. *Azospirillum brasilense*, another nitrogen-fixing bacterium, has been shown to produce plant growth substances[2,4]. Similarly *Azospirillum lipoferum* would have also produced auxins[6]. The increase in straw and grain yields due to the treatment was much more pronounced and all the eight cultivars responded almost equally well (table 1).

Variations in nitrogen-fixing ability of *Azospirillum* in different maize cultivars have been reported[10]. Varietal differences in nitrogen fixation in the rhizosphere of rice planted in pots have also been reported. However, Indu Bala and Kundu[11] observed that the wheat varieties tested by them uniformly responded to *Azospirillum* inoculation. The present results show that the yield response due to *Azospirillum* inoculation does not vary among the different rice varieties tested.

9 September 1988; Revised 1 December 1988

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ALTERATIONS IN AMINO ACID BALANCE IN RICE MUTANTS

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SINCE induction of mutation is the easiest and quickest method of rectification of genetic defects and improvement of a few traits in an otherwise well-adapted desirable variety, it was tried in rice using γ-rays, ethylmethane sulphonate (EMS) and diethyl sulphate (DES), singly and in combinations. Of the 396 mutants isolated from an M_2 population of 22,64,300 plants, the seeds of 14 mutants exhibited specifically patterned aminogram variability in M_4–M_7 generations. In the remaining 382 mutants, the variability was random and non-directional. Of the 14 mutants, five (Jm_1, Jm_4, IRm_1, IRm_6 and Bm_1) exhibited certain desirable phenotypic traits like shorter stature, earlier maturity, higher grain number or better grain-fineness than their corresponding initial lines (Kaul and Neelangini, unpublished). In addition, in all these mutants seed protein production was slightly increased. But the alterations in their essential amino acids are specific. These changes, termed amino acid balance alterations by Kaul[1], are described here.

For seed protein and amino acid analyses, four samples of 5 g of seeds of each mutant in each of the four generations were used. For amino acid analysis, 100 mg each of the four samples from each genotype were defatted with refluxing petroleum ether, hydrolysed under N_2 in 0.6 N HCl in a sealed tube at 40°C for 24 h, and subjected to amino acid analysis in a Spinco Model 120°C analyser with Spinco PA–35 and AA–5 resins[2]. Tryptophan was determined by PA–35 chromatography following the method of Knox *et al*[3]. The methionine content was determined similarly in AA–15 resin column as methionine sulphone after performing acid oxidation and hydrolysis with 0.6 N HCl[4]. The amino acid data were subjected to Duncan's multiple range test by the method outlined by Gomez and Gomez[5]. The seed protein content was estimated by the conventional micro-Kjeldahl's method using the established conversion factor 5.95. Estimates and significance of variability were computed following methods outlined by Steel and Torrie[6].

The mutants showed significant alterations in the levels of the essential amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (tables 1 and 2). Whereas isoleucine was significantly decreased in all the mutants, there was no significant alteration in tryptophan (table 2). Leucine, methionine and threonine were increased in Bm_1, IRm_1 and IRm_6, but both leucine and methionine were decreased in Jm_1 and Jm_4. The two mutants of Jhona and the two mutants of IR_8 showed similar amino acid alterations. One IR_8 mutant, IRm_1, and the Basmati mutant Bm_1 showed increase in seed protein content over their parents (table 2), but this enhancement was not proportional to increase or decrease of any individual amino acid (table 1). On the other hand, though there was no significant alteration in the seed protein content of the two Jhona mutants, Jm_1 and Jm_4, both of these showed significant decrease in isoleucine, leucine, methionine, phenylalanine, and increase in arginine and valine contents (table 2).

These findings indicate that amino acid alterations are independent of protein alterations in rice. This has also been found by many other workers[3, 7–9], but the presently reported directional alterations in specific amino acids were unknown in rice. Mutants exhibiting such alterations are termed amino acid

Table 1 *Essential amino acid content (g/16 g N) of rice seeds (parents and mutants)*

Amino acid	Bs	Bm_1	Jh	Jm_1	Jm_4	IR_8	IRm_1	IRm_6
Arginine	$8.7^a \pm 0.4$	$9.9^b \pm 0.5$	$9.0^a \pm 0.3$	$9.9^b \pm 0.3$	$9.9^b \pm 0.2$	$8.9^a \pm 0.2$	$7.5^b \pm 0.4$	$7.3^b \pm 0.3$
Histidine	$2.6^a \pm 0.3$	$2.0^b \pm 0.1$	$2.6^a \pm 0.5$	$2.8^a \pm 0.4$	$2.5^a \pm 0.5$	$2.7^a \pm 0.3$	$1.9^b \pm 0.3$	$1.4^b \pm 0.5$
Isoleucine	$4.5^a \pm 0.2$	$3.6^b \pm 0.4$	$4.6^a \pm 0.3$	$3.4^b \pm 0.2$	$3.5^b \pm 0.3$	$4.6^a \pm 0.2$	$3.9^b \pm 0.2$	$3.2^c \pm 0.3$
Leucine	$8.4^a \pm 0.3$	$9.4^b \pm 0.4$	$8.3^a \pm 0.2$	$7.0^b \pm 0.3$	$7.6^c \pm 0.2$	$8.4^a \pm 0.1$	$9.6^b \pm 0.5$	$9.8^b \pm 0.4$
Lysine	$3.8^a \pm 0.2$	$3.1^b \pm 0.2$	$3.7^a \pm 0.3$	$3.8^a \pm 0.3$	$4.1^a \pm 0.7$	$3.6^a \pm 0.4$	$3.3^a \pm 0.5$	$3.2^a \pm 0.3$
Methionine	$2.5^a \pm 0.1$	$2.9^b \pm 0.2$	$2.7^a \pm 0.2$	$2.1^b \pm 0.3$	$2.2^b \pm 0.2$	$2.8^a \pm 0.3$	$3.7^b \pm 0.2$	$3.8^b \pm 0.3$
Phenylalanine	$5.5^a \pm 0.3$	$4.5^b \pm 0.2$	$3.6^a \pm 0.2$	$3.9^a \pm 0.3$	$3.9^a \pm 0.3$	$3.6^a \pm 0.2$	$4.8^b \pm 0.5$	$4.8^b \pm 0.4$
Threonine	$3.5^a \pm 0.3$	$4.5^b \pm 0.2$	$3.6^a \pm 0.3$	$3.9^a \pm 0.3$	$3.9^a \pm 0.4$	$3.6^a \pm 0.3$	$4.8^b \pm 0.5$	$4.8^b \pm 0.6$
Tryptophan	$1.5^a \pm 0.2$	$1.4^a \pm 0.5$	$1.5^a \pm 0.3$	$1.4^a \pm 0.2$	$1.4^a \pm 0.4$	$1.5^a \pm 0.3$	$1.3^a \pm 0.5$	$1.3^a \pm 0.5$
Valine	$6.1^a \pm 0.3$	$6.2^b \pm 0.3$	$6.2^a \pm 0.2$	$7.6^b \pm 0.4$	$7.5^b \pm 0.5$	$6.0^a \pm 0.2$	$5.3^b \pm 0.3$	$5.2^b \pm 0.3$
Seed protein content (%)	$7.6^a \pm 0.11$	$8.3^b \pm 0.18$	$7.8^a \pm 0.25$	$7.9^a \pm 0.28$	$7.8^a \pm 0.17$	$7.6^a \pm 0.31$	$8.7^b \pm 0.16$	$7.2^a \pm 0.27$

Bs, Basmati; Bm_1, Basmati mutant; Jh, Jhona; Jm_1 and Jm_4, Jhona mutants; IR_8, dwarf rice; IRm_1 and IRm_6, dwarf rice mutants; All figures are mean ± SD; Mean values with different superscript letters (a, b, c) differ significantly from each other (across rows) at 5 *P* level by Duncan's multiple range test.

Table 2 *Alterations in essential amino acid content of seeds of rice mutants*

Mutant	Inducer mutagen	Amino acid alterations*			Protein content alteration
		Increase	Decrease	Nil	
Bm_1	EMS (0.5%)	arg, leu, met, thr	his, ile, lys, val	phe, trp	+
Jm_1	EMS (1.5%)	arg, val	ile, leu, met, phe	his, lys, thr, trp	Nil
Jm_4	γ-rays (20 kr) + EMS (1%)	arg, lys, val	ile, leu, met, phe	his, thr, trp	Nil
IRm_1	EMS (1%)	leu, met, thr	arg, his, ile, phe, val	lys, trp	+
IRm_6	EMS (1.5%)	leu, met, thr	arg, his, ile, phe, val	lys, trp	Nil

*Alterations significant at 5 *P* level; '+' indicates increase from level in corresponding initial line.

mutants. Whether such amino acid mutants exist in other cereals has not been fully established so far.

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CYTOMIXIS IN PMCs OF *SACCHARUM SPONTANEUM* L.

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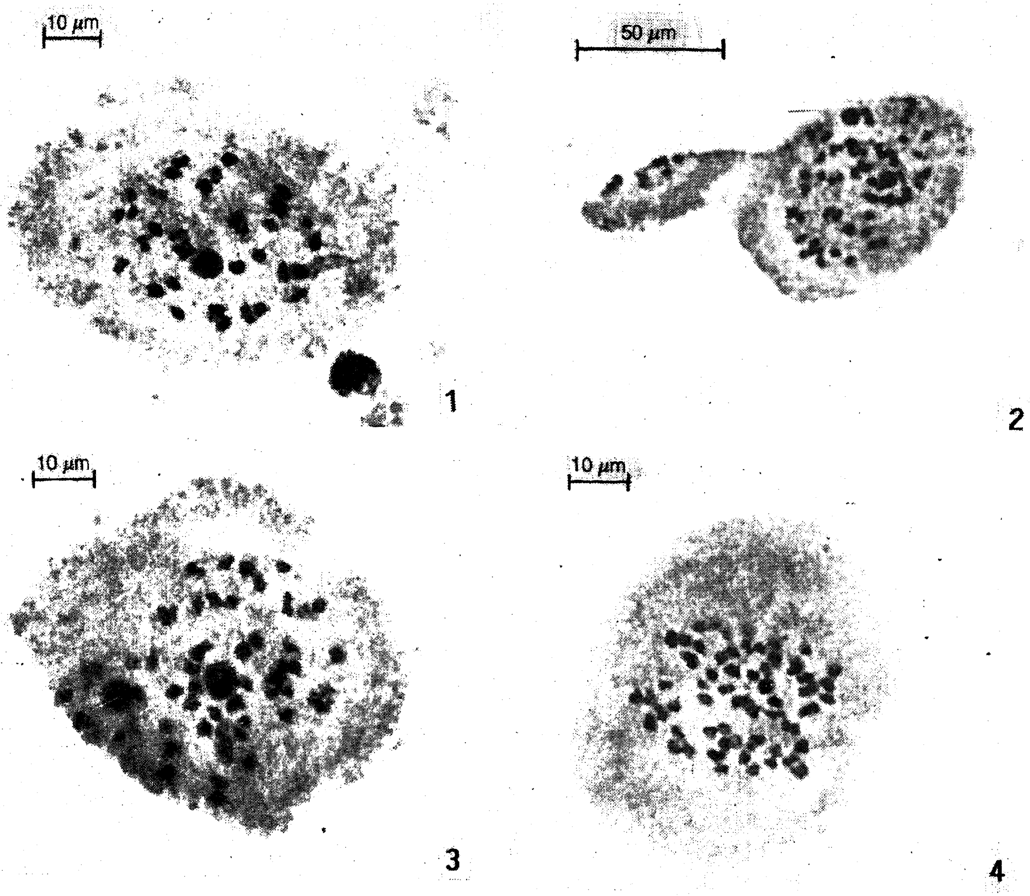
CYTOMIXIS, the transfer of nuclear material from one cell to the cytoplasm of an adjacent cell, is one of the ways for evolution of karyotype[1-8]. In a study of meiosis in various clones of *Saccharum spontaneum* L. (Poaceae) of Bhagalpur, cytomixis was observed in 20 pollen mother cells (PMCs) out of a total of 100. The phenomenon is hitherto unreported in this species.

The diploid chromosome number in the clone under study is 62 (usually as 31 bivalents, but sometimes as 30 bivalents plus 2 univalents), which is in conformity with the data available for the 'Central sector' of Panje and Babu[9]. The prophase-I nuclei contain a large nucleolus (figure 1), which vanishes during metaphase-I. In one case, two of the PMCs were seen to participate in cytomixis by the formation of a cytoplasmic channel, contributed, most probably, by the donor cell. Eight bivalents were still present in the donor, while the remaining 23 had already migrated to the recipient, bringing the total number of bivalents in the recipient to 54 from 31 (figure 2). The donor cell is diminutive owing to the outflow of its contents. In other cells, complete transfer of all the 31 bivalents appeared to have been accomplished (figure 3).

The probable time of such transfer (cytomixis) is premetaphase-I, as evidenced by the presence of two nucleoli in the recipient cell—one of the recipient itself, and the other obtained from the donor (figures 2 and 3). In metaphase-I of such a syndiploid (figure 4), both the nucleoli disappeared, and the bivalents appeared to be oriented on two separate plates. The mean volume (calculated after Uhl and Moran[10]) of the diploid PMCs is 1.3×10^4 μm^3 and that of the syndiploid ones 2.5×10^4 μm^3 (table 1).

The 1.9 fold increase in the volume of the recipient



Figures 1–4. Cytomixis in PMCs of *S. spontaneum*. **1,** Prophase-I nucleus with a large nucleolus. **2,** Two PMCs undergoing cytomixis through a cytological channel in between. **3,** A recipient cell during pre-metaphase with two nucleoli and double the number of bivalents. **4,** A syndiploid in metaphase-I.

Table 1 *Size of diploid and syndiploid PMCs*

Parameter	Mean value	
	Diploid	Syndiploid
Longest semidiameter (μm)	18.65	23.27
Semidiameter perpendicular to longest semidiameter (μm)	13.25	16.25
Volume (μm^3)	1.3×10^4	2.5×10^4

cell cannot be due to addition of chromosomes alone. Though no empty or anucleate 'ghost cells' described earlier[3,5], were seen, flow of donor cytoplasm into the recipient is likely to have taken place[1].

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ON THE OCCURRENCE OF A NEW HEXAPLOID CYTOTYPE OF *COIX LACRYMA-JOBI* LINN. FROM NORTH-EAST INDIA

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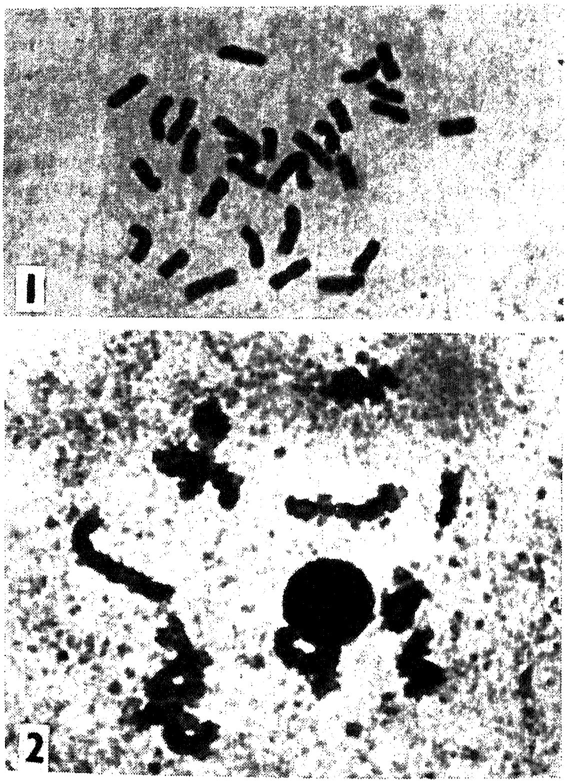
THE genus *Coix* of the tribe Maydeae of the family Gramineae consists of four species[1]. *Coix lacryma-jobi* is polymorphic with many varieties and is cosmopolitan in distribution. A perusal of the literature revealed that this genus shows different levels of polyploidy, viz. diploids ($2n=10$), tetraploids ($2n=20$) and octoploids ($2n=40$)[2-4]. A few aneuploids have also been reported[5,6]. However, no hexaploid species, with $2n=30$, has so far been reported. During an extensive collection of this genus from different parts of India, *C. lacryma-jobi* var. *ma-yuen* was collected from hilly state of Meghalaya. This species is cytologically interesting as it showed the hexaploid somatic number, $2n=30$. This is the first report of a hexaploid cytotype in *Coix*.

The seeds of *C. lacryma-jobi* var. *ma-yuen* were collected from the Khasi Hills, Meghalaya, where it is cultivated by the hill tribes. The fruit-case is soft, thin-shelled, spheroidal, white in colour and about 0.7 cm in diameter. The plants were raised in the botanical garden of this University for cytological studies. Chromosome studies were made from pollen mother cells and root-tip cells fixed in 1:3 acetic alcohol and stained in aceto-carmine.



Figures 1 and 2. **1,** Metaphase of mitosis, $2n=30$ (× 1750). **2,** Diakinesis of meiosis (× 1750).

Aneuploid; Quadrivalent

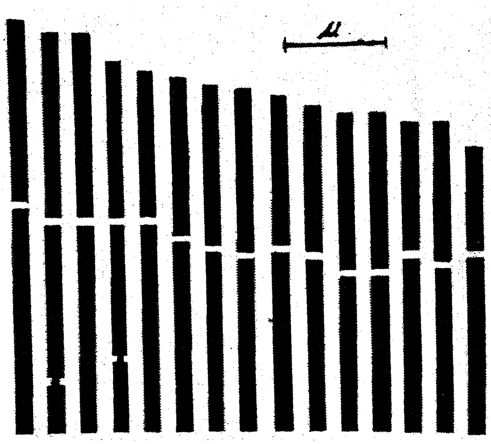


Figure 3. Idiogram of *C. lacryma-jobi* var. *ma-yuen* ($2n=30$).

The somatic chromosome number was consistently found to be $2n=30$ in the root-tip cells (figure 1). Karyotype analysis showed that the length of the chromosomes ranges from 2.66 to 3.91 μm with two pairs of M-type and 13 pairs of m-type and of 1A category[7]. Two pairs of chromosomes possess a secondary constriction in the long arm distal to the centromere (figure 3). It may be noted that the chromosomes of this taxon are similar to the other varieties of *C. lacryma-jobi* in size and karyotype category. Meiosis was found to be markedly disturbed, and at metaphase I, 3–5 quadrivalents were frequently observed along with bivalents (figure 2). Two bivalents or sometimes a single quadrivalent were found commonly associated with the nucleolus. Pollen sterility was found to be about 45%. *C. lacryma-jobi* is presumably of hybrid origin.

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DIFFERENTIAL RADIATION SENSITIVITY IN MOTH BEAN

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MOTH bean, *Vigna aconitifolia* (Jacq.) Marechal, comprises one of the important pulse species of India. Being an extremely drought-resistant crop, it is grown widely in the arid and semiarid zones of Rajasthan, Gujarat, Maharashtra, Haryana and Uttar Pradesh.

Growing only 20–25 cm tall, the plant forms a mat across the soil surface. The stem of each plant radiates horizontal branches, producing an expanding circlet of densely matted, ground-hugging vegetation. A living mulch, moth bean shields soil from the sun's heat and retards soil erosion. Livestock avidly graze on its pods and foliage. The pods when young are used as a table vegetable. They contain tiny beans which are rich in proteins and other nutrients. The plant as a whole is a good source of quality forage under arid and semiarid conditions.

Although the plant has multifaceted importance, it has received only scant attention regarding its genetic improvement. Therefore it was thought worthwhile to look into the possibilities of improving moth bean through induced mutation. The present paper deals with evaluation of varietal radio-response in moth bean based on morphological parameters in R_1 and R_2 generations.

For recording the radio-response, dry seeds of three moth bean varieties, viz. Local, IPCMO 186 and MG-1, of uniform size and moisture content, were irradiated with different doses of gamma rays (5–25 kR). The irradiated seeds were sown in the field in randomized block design with three replications and the R_1 generation was raised. The effect of gamma rays on morphological parameters such as germination, survival of plants, plant height and commencement of flowering was studied. From the seed progeny of the R_1 generation, the R_2 generation

Table 1 *Relative sensitivity of moth bean varieties to gamma rays in R_1 generation*

Variety	Dose (kR)	Germination (% of control)	Survival (% of control)	Plant height (cm)	Commencement of flowering (days)
Local	Control	—	—	23.00 ± 0.10	80.00 ± 0.11
	5	103.42	103.05	27.00 ± 0.12	76.00 ± 0.15
	15	96.96	97.73	21.70 ± 0.18	82.00 ± 0.12
	25	90.72	89.36	19.25 ± 0.21	84.00 ± 0.19
IPCMO 186	Control	—	—	20.50 ± 0.15	72.00 ± 0.25
	5	101.13	101.06	22.15 ± 0.11	70.00 ± 0.22
	15	97.28	94.68	18.20 ± 0.13	74.00 ± 0.28
	25	93.27	87.23	17.05 ± 0.17	76.00 ± 0.15
MG-1	Control	—	—	23.00 ± 0.18	65.00 ± 0.35
	5	98.58	97.87	21.80 ± 0.20	66.00 ± 0.31
	15	95.86	96.72	19.75 ± 0.17	69.00 ± 0.32
	25	92.13	93.08	19.30 ± 0.15	72.00 ± 0.24

Figures in the last two columns are mean ± SE.

Table 2 *Relative sensitivity of moth bean varieties to gamma rays in R_2 generation*

Variety	Dose (kR)	Germination (% of control)	Survival (% of control)	Plant height (cm)	Commencement of flowering (days)
Local	Control	—	—	23.10 ± 0.11	80.00 ± 0.10
	5	104.50	103.00	27.23 ± 0.15	77.00 ± 0.20
	15	97.15	98.10	22.00 ± 0.21	81.00 ± 0.15
	25	91.23	90.12	20.00 ± 0.22	84.00 ± 0.11
IPCMO 186	Control	—	—	20.61 ± 0.14	72.00 ± 0.12
	5	102.00	102.00	22.75 ± 0.10	73.00 ± 0.26
	15	97.51	95.15	18.65 ± 0.14	74.00 ± 0.30
	25	94.00	88.00	17.50 ± 0.18	76.00 ± 0.15
MG-1	Control	—	—	23.05 ± 0.11	65.00 ± 0.35
	5	98.75	98.15	21.95 ± 0.19	67.00 ± 0.32
	15	96.12	97.80	20.00 ± 0.18	69.00 ± 0.30
	25	93.00	94.00	19.55 ± 0.17	71.00 ± 0.28

Figures in the last two columns are mean ± SE.

was grown. The R_2 plants were examined for effects of gamma radiation.

The results are given in tables 1 and 2. There was a general exponential fall in germination, survival and plant height with increasing gamma ray doses in both R_1 and R_2 generations. The period (days) for commencement of flowering increased with gradual increase in radiation doses. At 5 kR, a slight stimulatory effect was quite evident in varieties Local and IPCMO 186. In the case of variety MG-1, however, such a trend was not detectable.

There are several reports of differential response of varieties to radiation[1-3]. Radiosensitivity differences between varieties and species have been ascribed to moisture content of seeds[4,5], maturity and structure of embryo[6], the cell nucleus[7], the size and structure of the chromosome complement[8], and nuclear volume and ploidy level[9].

Mutagenic sensitivity is believed to be influenced by environmental and biological factors[10]. Variations of physiological conditions among individuals of the same variety have been shown to affect radiosensitivity[11]. The genetic background has been implicated in the differential mutagenic response of seeds[12]. Genetic control of intervarietal variations in mutagen sensitivity has been very well documented[13]. Varieties are believed to differ not only in their morphological characters but also in their genetic architecture, which could act as a potent factor in determining their response to mutagens[14].

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NEUROENDOCRINE CONTROL OF PROTEIN AND AMINO ACIDS IN THE TISSUES OF FRESHWATER CRAB, *BARYTELPHUSA GUERINI* (H. MILNE EDWARDS) (DECAPODA, POTAMIDEA)

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STUDIES on *Hemigrapsus nudus* showed that loss of eyestalk factor through removal of sinus gland led to acceleration of catabolic processes in the tissues[1]. Eyestalk removal led to decrease in total nitrogen content, amylolytic activity and RNA content of *Procambarus clarkii*[2] and *Barytelphusa cunicularis*[3], while eyestalk injections into eyestalk-ablated animals increased RNA levels. Histochemical studies on the hepatopancreas of *Scylla serrata* showed that glycoproteins and glycolipids were depleted following eyestalk ablation[4]. Variations in free amino acid levels in relation to eyestalk factors were also noticed in a few crustaceans[5–7]. To understand the neuroendocrine control of protein and amino acid levels in the tissues of the freshwater crab *Barytelphusa guerini*, the effects of eyestalk ablation, eyestalk injection into ablated animals, and other neuroendocrine structures were studied.

The collection and maintenance of the freshwater crab *B. guerini* were described earlier[8]. Males weighing between 30 and 50 g were used for the experiments, carried out at room temperature (26–28°C). The total protein and amino acid content of tissues like muscle, gill, heart and hepatopancreas were estimated quantitatively[9,10] in normal animals with intact eyestalks. Another group of laboratory-adapted crabs was employed for eyestalk ablation experiments. Forty-eight hours after eyestalk ablation, the animals were divided into five batches. One batch of eyestalk-ablated animals was used as control ablated animals and total protein and amino acid content were estimated. Animals in the remaining four experimental batches were given injections of extracts of eyestalks, sinus gland, brain and thoracic ganglionic mass respectively. Protein and amino acids were estimated 48 h after extract injection.

Eyestalk ablation and preparation of neuroendocrine extracts were done according to the procedures described earlier[8]. Total protein was determined[9] and the amino acids were quantified[10] by using 1 ml of 1% homogenates of tissues prepared in 0.025 M sucrose solution. At least 6 animals were used for each determination and the results were statistically analysed using Student's *t* test.

Figure 1 shows the results for total protein of muscle, gill, heart and hepatopancreas. Total protein was increased by 44.39% in muscle, 62.28% in gill and 57.32% in heart 48 h after eyestalk ablation. In each case the increase is highly significant ($P < 0.02$). Injection of eyestalk extract into eyestalk-ablated animals brought down protein content to the normal level in about 48 h. The recovery was almost complete, and the difference in protein content between animals that received eyestalk extract and

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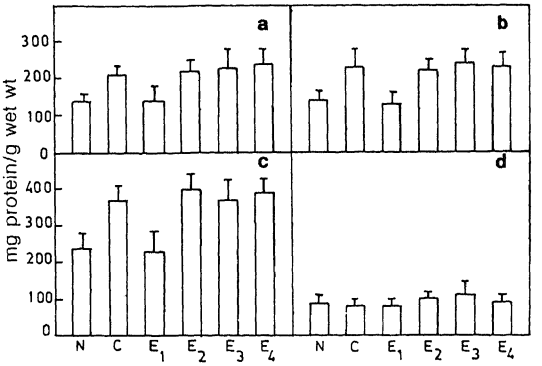

Figure 1. Total protein content of muscle (**a**), gill (**b**), heart (**c**) and hepatopancreas (**d**) of *B. guerini*, in normal, intact animals (N), after eyestalk ablation (C), and in ablated animals after injection of extract of eyestalk (E_1), sinus gland (E_2), brain (E_3) and thoracic ganglionic mass (E_4). Bars show means of 6 determinations.

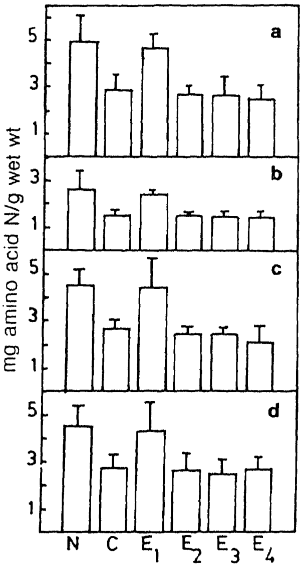

Figure 2. Amino acid content of muscle (**a**), gill (**b**), heart (**c**) and hepatopancreas (**d**) of *B. guerini*, in normal, intact animals (N), after eyestalk ablation (C), and in ablated animals after injection of extract of eyestalk (E_1), sinus gland (E_2), brain (E_3) and thoracic ganglionic mass (E_4). Bars show means of 6 determinations.

normal animals was insignificant ($P > 0.1$). In the hepatopancreas, however, protein content was increased by about 8.25% 48 h after eyestalk ablation. This change is not significant ($P > 0.1$). Injection of eyestalk extract into eyestalk-ablated animals did not produce any significant change in protein content of hepatopancreas.

Amino acid content was decreased by 43.57% in muscle, 44.85% in gill, 41.70% in heart and 39.77% in hepatopancreas 48 h after eyestalk ablation (figure 2). This decrease is also highly significant ($P < 0.02$). Injection of eyestalk extract into eyestalk-ablated animals elevated amino acid content to the normal level in about 48 h. Again, the recovery was almost complete, the difference in amino acid content between extract-injected animals and normal animals being insignificant ($P > 0.1$).

Injection of extracts of sinus gland, brain and thoracic ganglionic mass into eyestalk-ablated animals did not reverse the effects of ablation in any of the four tissues (figures 1 and 2). Eyestalk ablation did not produce significant change in total protein content of hepatopancreas (figure 1), but caused a decrease in amino acid content, as in the other tissues examined (figure 2).

Earlier studies on *Astacus astacus*[5] and *Callinectes sapidus*[6] showed that eyestalk ablation facilitates the incorporation of amino acids into tissue proteins and their utilization for metabolic energy release. It was suggested earlier that, in *B. guerini*, decreased blood sugar level after eyestalk ablation is possibly due to stimulation of glycogenesis[11]. It has also already been reported that decreased amino acids in the blood after eyestalk ablation may be due to their utilization for energy release in the absence of sugars, because the sugars are utilized in the process of lipid synthesis and glycogenesis[12]. There is also evidence to show that eyestalk ablation leads to increased lipid synthesis and gluconeogenesis[4, 13–15]. The utilization of amino acids as an important source of energy-producing compounds has been well demonstrated in crustaceans[16]. The present results suggest the possible role of eyestalk factor in regulating protein and amino acid content of tissues. It appears that the eyestalk contains a factor that inhibits incorporation of amino acids into tissue proteins. Thus the present study provides good evidence for a protein synthesis inhibitory factor in the eyestalk of *B. guerini*. This view is further strengthened by the fact that eyestalk removal in *Orconectes virilis* in premoult stages produced a greater incorporation of amino acid into tissue proteins in all tissues[7].

Studies on *H. nudus*[1], *P. clarkii*[2], *B. cunicularis*[3], *S. serrata*[4] and *Palaemon vulgaris*[17] showed a significant decrease in the protein content of hepatopancreas. The absence of any such significant change in the present study may perhaps be due to the fact that RNase and RNA content of hepatopancreas in *B. guerini* are less susceptible to the action of eyestalk hormone, and therefore the protein synthetic rate is not adversely affected. Alkaline phosphatase activity in hepatopancreas of *Astacus leptodactylus* did not change significantly after sinus gland removal[18].

Sinus gland extract did not restore protein and amino acid levels, suggesting that the factor influencing protein metabolism is not present in the sinus gland. It is possible that the factor produced in the X-organ complex of the eyestalk is released directly into the blood stream instead of being stored in the sinus gland[19]. Central nervous system structures also did not affect protein and amino acid content, suggesting the absence of any factors influencing protein metabolism in these structures. The present study thus clearly shows that total protein and amino acid content of tissues in *B. guerini* are under the control of a factor in the eyestalk. It is also evident that the eyestalk factor shows some tissue specificity.

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A REPORT ON THE PRESENCE OF VARIOUS PATHOGENIC MICROBES IN A WILD POPULATION OF BIHAR HAIRY CATERPILLAR, *DIACRISIA OBLIQUA*

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BIHAR hairy caterpillar, *Diacrisia obliqua*, is one of the most serious pests on mulberry plantations, and harbours many pathogens[1-3] common to the silkworm *Bombyx mori* and serves as a reservoir host. Cross-infectivity of *Nosema bombycis* and *Borrelina* virus between *Diacrisia obliqua* and *Bombyx mori* has already been reported[4]. The present study was carried out to find out the presence of pathogenic microbes in *D. obliqua*.

Larvae of *D. obliqua* were collected from the mulberry field of this Institute. One thousand larvae each of II, III, IV, V, VI and VII instars were examined for the presence of various pathogenic microbes. Tissues examined were haemolymph and midgut. Per cent incidence of various pathogens is presented in table 1.

Polyhedra; Nosema sp

Table 1 *Per cent incidence of various pathogens recorded in field populations of Diacrisia obliqua*

Instar	Per cent incidence				
	Nosema sp.	Polyhedra	Bacteria	Double infection with polyhedra and bacteria	No pathogen
II	0	43.29	10.43	15.28	31.00
III	7.57	43.57	7.14	13.15	28.57
IV	0	32.71	23.86	21.14	22.29
V	0	32.83	30.67	19.83	16.67
VI	0	33.86	27.30	18.56	20.28
VII	0	45.80	18.40	14.60	21.20

The data show that *Nosema* sp. was noticed only in 7.57% of III instar larvae, but was not found in other instars. Viral polyhedral bodies were more frequently encountered in all the instars. Incidence of bacteria shows considerable variation in the different instars. Double infection with viral polyhedra and bacteria was also recorded, and ranged between 13.15 and 21.14% in the different instars. In all instars, larvae free from infection were also encountered.

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SERUM LIPID PROFILES IN CRYPTORCHID ALBINO RATS

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CRYPTORCHIDISM is a common (often endocrine) disorder[1,2]. Scrotal insulation and artificial cryptorchidism cause sterility in man and animals[3]. Cryptorchidism induces decrease in size and weight of testis and causes atrophy of the testis as well as the accessory reproductive organs of the rat[2,4–6]. In man, cryptorchidism is associated with azoospermia or oligospermia with poor spermograms[7]. In cryptorchid animals, the serum gonadotrophin levels are significantly elevated[8,9], with decreased testosterone titre[10]. In view of the changes in the plasma hormonal levels and physiological derangement during cryptorchidism, it is worthwhile to undertake studies on serum lipid fractions after cryptorchidism.

A total of 20 Wistar albino rats (100 days old, 150 ± 5 g body weight) were selected for the study. The rats were divided into two groups of 10 each. One group was subjected to bilateral cryptorchidism as described earlier[5]. The second group was sham-operated and used as control. Both groups were maintained under the same laboratory conditions (26 ± 2°C, 12:12 dark : light periods), fed on balanced rat pellet diet (Hindustan Lever Ltd, Bombay), and given water *ad libitum*. One month after the operation the animals were sacrificed by cervical dislocation and the blood was collected. Heparinized blood was centrifuged and the serum was collected and stored (−2°C) until use.

Total lipid[11], phospholipids[12], lipase activity[13], free fatty acids, cholesterol, triglycerides[14], and glycerol[15] were estimated by the usual methods.

Table 1 shows the changes in serum lipids associated with cryptorchidism in albino rats. The normal serum lipid values obtained in the present study conform with those of previous studies[16].

The total lipid content of the serum was decreased in cryptorchid rats, indicating increased lipolysis. Hence lipase activity was determined and found to be markedly elevated in cryptorchidism. Triglycerides decreased significantly with the elevation in lipase activity. The increase in lipolysis also elevated the

Table 1 *Levels of total lipids, phospholipids, free fatty acids, triglycerides, glycerol and cholesterol (mg/100 ml), and lipase activity (μmol of PNRA cleaved/mg protein/h) in serum of control and cryptorchid rats*

Component	Control	Cryptorchid	Change over control (%)
Total lipids	487.98 ± 13.48	403.52 ± 10.89	− 17.30 $P < 0.001$
Phospholipids	71.68 ± 2.81	54.8 ± 3.11	− 23.55 $P < 0.001$
Lipase activity	0.029 ± 0.001	0.043 ± 0.001	+ 48.28 $P < 0.001$
Free fatty acids	63.51 ± 2.83	39.92 ± 2.04	− 37.14 $P < 0.001$
Triglycerides	9.54 ± 0.68	5.17 ± 0.30	− 45.81 $P < 0.001$
Glycerol	14.83 ± 0.92	20.66 ± 1.30	+ 39.31 $P < 0.001$
Cholesterol	78.19 ± 3.45	93.88 ± 3.14	+ 20.07 $P < 0.001$

Values are mean ± SD ($n = 10$).

glycerol content of serum. But free fatty acids were decreased, suggesting their mobilization into oxidative metabolism and ketogenesis[17]. Hence in cryptorchidism serum lipid metabolism seems to be oriented towards lipid oxidations resulting in decreased triglycerides. Phospholipids were lower in cryptorchid animals, indicating increased uptake of phospholipids by the testis and accessory reproductive organs. The cryptorchid testis is known to preferentially utilize lipid components, particularly the phospholipid fractions[5]. The increase in serum cholesterol in cryptorchid animals indicates active addition of cholesterol into the blood from the synthetic sites and/or decreased uptake by tissues. Hence accelerated synthesis of cholesterol in tissues of cryptorchid animals can be expected.

In general, the results suggest that short-term cryptorchidism leads to effective depletion of some lipid components from the serum and an accumulation of cholesterol.

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NITRATE REDUCTASE FROM THE MARINE CYANOBACTERIUM *OSCILLATORIA LAETEVIRENS* (CROUAN) GOM.

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THE enzyme nitrate reductase catalyses the first step in the assimilation of nitrate, namely the reduction of nitrate to nitrite. In the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp., the effect of nitrogen source on the activity of assimilatory nitrate reductase has been studied. In these species, nitrate behaves as a nutritional inducer of nitrate reductase[1–7], and enzyme activity is repressed during growth on ammonia[1]. The growth rate of the marine cyanobacterium *Oscillatoria laetevirens* (Crouan) Gom is related to the source and concentration of nitrogen supplied. Cultures that received nitrate showed higher growth rate than those that received ammonia and nitrite[8]. Studies on uptake kinetics have revealed that the culture has high affinity for nitrate[8]. Hence it was of interest to study nitrate reductase in nitrate-grown marine *O. laetevirens*.

O. laetevirens was isolated from coral stone collected from the intertidal region of the Porbandar coast (21°38′ N, 69°37′ E). The culture was grown using seawater medium under controlled conditions, as described earlier[8].

Nitrate reductase activity was assayed by the method of Lowe and Evans[9]. A known amount of fresh algal culture mass was washed 3–4 times with sterile distilled water containing 2.5% NaCl and then incubated in sterile culture medium containing 5 mM KNO_3, pH 7.5. Samples were taken after 18 h of incubation in controlled conditions, and the nitrite formed was measured by a diazo coupling method. Absorbance at 540 nm of the pink colour developed was measured after 15 min. The reagent blank was 1 ml medium instead of culture supernatant. $NaNO_2$ was used as standard. Protein content of the algal culture was estimated by the method of Lowry *et al.*[10] using bovine serum albumin as standard.

Table 1 shows nitrate reductase activity of *O. laetevirens* in culture. A gradual increase in the enzyme activity was seen up to 11 days of incubation, and then there was no significant change in the activity. Cultures were grown in different concentrations of nitrate and enzyme activity was determined on day 11. The enzyme gave a Michaelis–Menten pattern (figure 1a). The Lineweaver–Burk plot gave K_m 0.181 mM and V_{max} 11.11 nmol/mg protein (figure 1b).

Table 1 *Nitrate reductase activity in marine O. laetevirens in culture*

Days of incubation	Nitrate reductase activity (nmol NO_2 produced/mg protein)
4	4.675
8	7.400
11	11.800
16	9.650

In higher plants the role of nitrate as an inducer of the enzyme has been well studied[11–15]. Increase in the level of nitrate reductase in the presence of nitrate implies *de novo* synthesis of the enzyme[13–15]. Studies with cycloheximide and other inhibitors of protein synthesis have shown that in both higher plants and eukaryotic algae, the enzyme is synthesized on 80 S cytoplasmic ribosomes[16–23]. Nitrate reductase activity of the prokaryotic *O. laetevirens* grown in the presence of the protein synthesis inhibitor chloramphenicol was studied. At 6 μg/ml chloramphenicol added at the start of incubation, there was an inhibition of growth. When the antibiotic was added after two days of growth enzyme activity fell

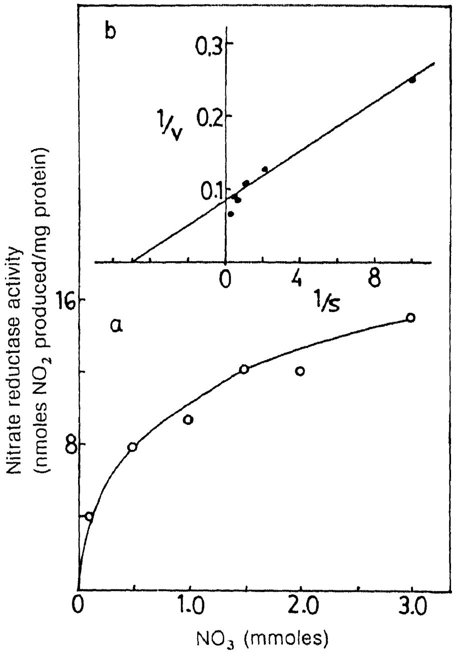

Figure 1. Substrate concentration vs velocity (**a**) and Lineweaver–Burk (**b**) plots for nitrate reductase activity in *O. laetevirens*.

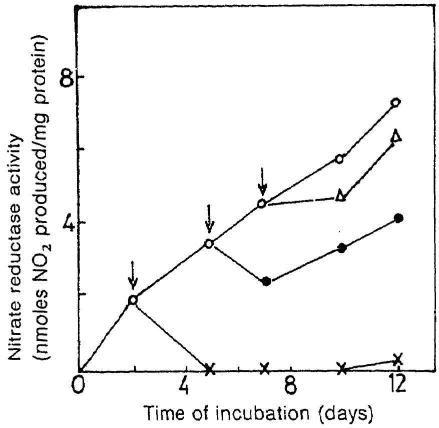

Figure 2. Effect of chloramphenicol on nitrate reductase activity in *O. laetevirens*: chloramphenicol absent (O–O), or added on day 2 (×–×), day 5 (●–●) or day 7 (△–△) of culture.

to zero by day 5 and was totally absent up to day 12; and then only 3.57% of the activity of the control culture could be observed (figure 2). When the antibiotic was added to five-day-old and seven-day-old cultures, about 55% and 85% enzyme activity respectively could be detected on day 12. These results indicate that most of the enzyme protein might be synthesized in the early stage of incubation, i.e. within the first five days. During this period the enzyme was sensitive to antibiotic-mediated inhibition. Once the rise in enzyme activity commenced, severity of inhibition was less. The results are similar to those for the eukaryotic *Chlorella vulgaris*, where cycloheximide inhibits the development of nitrate reductase activity only when added immediately after the transfer of cells from ammonia to nitrate medium, but not when added after the rise in enzyme activity has commenced[17].

The present results show that nitrate reductase of *O. laetevirens* is an inducible enzyme.

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METABOLIC RESISTANCE TO METHYL PARATHION TOXICITY IN A BIVALVE, *LAMELLIDENS MARGINALIS*

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INDISCRIMINATE use of pesticides is causing serious ecological imbalance, particularly in freshwater

Pesticide toxicity :

Table 1 *Metabolic changes in mussels exposed to methylparathion (MP) for one to four weeks*

		1 week		2 weeks		3 weeks		4 weeks	
		Control	MP	Control	MP	Control	MP	Control	MP
Total protein (mg/g wet weight)	Foot	59.045 ± 3.73	43.397* ± 6.82 (−26.50)	59.412 ± 9.81	34.317* ± 9.52 (−42.23)	57.647 ± 13.11	49.60 ± 7.88 (−13.95)	57.831 ± 11.82	33.837* ± 5.99 (−41.48)
	Mantle	41.910 ± 14.77	35.930 ± 12.07 (−14.26)	42.687 ± 11.62	38.647 ± 9.26 (−9.46)	41.972 ± 11.45	32.872 ± 3.28 (−21.68)	40.201 ± 7.79	29.281* ± 5.30 (−27.16)
Free amino acids (μmoles of tyrosine equivalents/g wet weight)	Foot	79.15 ± 13.52	106.49* ± 10.49 (+34.54)	81.67 ± 7.38	127.24* ± 19.97 (+55.79)	83.88 ± 11.92	85.53* ± 11.49 (+1.967)	69.64 ± 2.77	115.66* ± 10.95 (+66.08)
	Mantle	82.44 ± 15.59	72.95 ± 6.79 (−11.51)	79.86 ± 10.09	72.29 ± 7.76 (+9.48)	82.29 ± 7.76	81.82 ± 6.39 (−0.57)	75.04 ± 6.43	102.27* ± 22.21 (+36.28)
Glutaminase (μmoles of NH_3 formed/mg protein/h)	Foot	0.0918 ± 0.064	0.1088* ± 0.008 (+18.51)	0.0958 ± 0.008	0.1139* ± 0.008 (+18.89)	0.0979 ± 0.009	0.1186* ± 0.005 (+21.14)	0.0992 ± 0.012	0.1258* ± 0.006 (+26.81)
	Mantle	0.1327 ± 0.007	0.1486* ± 0.006 (+11.98)	0.1378 ± 0.008	0.1646 ± 0.003 (+19.44)	0.1379 ± 0.011	0.1755* ± 0.006 (+27.26)	0.1347 ± 0.009	0.1539* ± 0.007 (+14.25)
Catalase (μmoles of H_2O_2 formed/mg protein/h)	Foot	5.890 ± 0.43	6.633* ± 0.47 (+12.61)	6.106 ± 0.43	9.923* ± 0.28 (+62.51)	5.626 ± 0.71	10.529* ± 0.46 (+87.14)	5.175 ± 0.40	11.371* ± 0.43 (+119.73)
	Mantle	8.449 ± 0.30	9.476* ± 0.46 (+12.15)	8.816 ± 0.30	12.818* ± 0.34 (+45.39)	8.844 ± 0.25	16.819* ± 0.48 (+90.17)	8.633 ± 0.40	10.713* ± 0.38 (+24.09)
Xanthine oxidase (μmoles of formazone formed/mg protein/h)	Foot	0.1387 ± 0.010	0.1808* ± 0.09 (+30.35)	0.1432 ± 0.04	0.2159* ± 0.01 (+50.76)	0.1323 ± 0.02	0.2347* ± 0.01 (+77.39)	0.1421 ± 0.03	0.1658* ± 0.08 (+10.67)
	Mantle	0.1063 ± 0.08	0.1259* ± 0.02 (+18.43)	0.1090 ± 0.01	0.1522* ± 0.08 (+39.63)	0.1129 ± 0.01	0.1930* ± 0.01 (+70.94)	0.1025 ± 0.09	0.1731* ± 0.02 (+68.87)

Values are mean ± SD ($n = 8$); figures in parentheses are per cent change over control.
*Significant difference, $P < 0.05$.

ecosystems. Invertebrates constitute a very large proportion of the fauna in aquatic ecosystems. Freshwater mussels have been commonly employed in toxicity evaluation studies and water quality management programmes[1-3]. In the present study the biochemical and physiological responses to methylparathion (MP) toxicity with reference to ammonia detoxification has been evaluated in the tissues of a bivalve to assess the tissue-specific metabolic compensatory mechanisms that aid survival chances of mussels.

Bivalves *Lamellidens marginalis* (25 ± 5 g) were acclimated to laboratory conditions in aquaria under 12:12 light:dark periods. The physicochemical characteristics of water were: temperature 27 ± 2°C, pH 7.1–7.3, hardness 61 mg/l (HCO_3^-), dissolved oxygen 5.3 ± 0.72 ml/l.

Based on dose–mortality and tolerance data, a pesticide level of 15 ppm (sub-lethal concentration) was selected. The animals were divided into two batches of 20 each and one batch was exposed to 15 ppm of MP (obtained from Pesticides India Ltd, Bombay) for four weeks. The water in the troughs was changed every 24 h. A second group of animals maintained under identical conditions in freshwater (without MP) served as control.

After the stipulated time (1, 2, 3 and 4 weeks) foot and mantle from both control and MP-exposed mussels were excised and chilled to 0°C. Total protein[4], free amino acids (FAAs)[5], catalase[6], xanthine oxidase[7,8] and glutaminase[9] were estimated.

Table 1 gives the results of the study. MP was reported to trigger acid and alkaline protease activity of the tissues[3,10]. Seshagiri Rao[11] reported histopathological alterations in foot during pesticide stress. These observations point to augmented tissue protein degradation, which might be responsible for the reduction in tissue proteins under MP toxicity. The unique metabolic potential of mantle[12] and its defensive action, viz. secretion of mucus[13], might be responsible for the smaller decrease in total protein content compared to foot (table 1).

Increased proteolysis is always associated with high FAA levels. The FAAs may be used for the synthesis of specific enzymes[14]. In order to cope with the toxic condition, they may be channelled to the TCA cycle to meet the energy demand[15] or to the regulation of ionic and osmotic balance. The FAAs may also contribute to gluconeogenesis and keto acid formation. The rise in tissue FAA levels during MP stress might be a compensatory mechanism by which these FAAs provide energy through transdeamination during stress to meet the high energy demand.

The deamination of FAAs results in the production of peroxides[16]. The increase in catalase activity in the tissues of MP-exposed mussels indicates possible elevation of amino acid oxidase activity and predominance of deamination resulting in more peroxide formation. Such a metabolic situation warrants enhanced catalase activity to mobilize peroxides.

Glutamine oxidation was found to increase in response to the energy demand in the tissues under stress conditions. Since metabolic acidosis was reported during MP stress in the tissues of freshwater mussels[17], stepped-up ammonia production by increased glutaminase activity may be an adaptive measure to maintain the acid–base balance. The high glutaminase activity is indicative of rapid turnover of glutamine, a major product of ammonia. During pesticide stress the enzyme has to deal with increasing quantities of transported ammonia to maintain metabolic homeostasis.

Xanthine oxidase plays an important role in ammonia detoxification and nitrogen balance of the tissue. The increased activity of this enzyme in the tissues of MP-exposed mussels indicates increased synthesis of uric acid under toxic stress.

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IN VITRO DIGESTIBILITY OF CASHEW KERNEL PROTEIN

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PROTEIN content of defatted kernel flour of some cashew varieties has been shown to vary between 32.1 and 43.7%[1]. Cashew kernel protein contains all the essential amino acids[2,3]. Protein efficiency ratio of cashew-nut extraction meal (3.23) compares well with that of casein[4] (3.12). Lysine content of cashew kernel protein was significantly different among varieties[5]. Detailed studies were initiated to compare the varieties for *in vitro* digestibility of kernel protein by proteolytic enzymes such as trypsin, α-chymotrypsin and pepsin.

Trypsin (from bovine pancreas, 13,000 BAEE units/mg protein), α-chymotrypsin (from bovine pancreas, 47 units/mg protein) and pepsin (from porcine stomach mucosa, 3165 units/mg protein) were obtained from Sigma Chemical Co., USA. All the buffer salts used were of analytical reagent grade. Cashew kernel flour, after defatting with a chloroform–methanol mixture (2:1 v/v), was extracted with double-distilled water (1:20 w/v) at pH 10 and 4 for 60 min at 25°C and the supernatant was precipitated with ammonium sulphate (90% saturation). The precipitate was taken in a suitable buffer (0.1 M potassium phosphate, pH 7.6, for trypsin and α-chymotrypsin; 0.2 M KCl–HCl, pH 2.0, for pepsin) and dialysed overnight at 25°C against the same buffer. Protein in the extract was estimated by Bradford's dye method[6].

In vitro digestibility of cashew kernel protein by trypsin, α-chymotrypsin and pepsin was studied as described earlier[7]. The ratio of enzyme to protein was 1:100. Protein for the assay varied between 3 and 3.5 mg/ml, and the enzyme concentration was 35 μg/ml. For pepsin, kernel protein and enzyme were taken separately in 1 ml of 0.2 M KCl–HCl buffer (pH 2.0), while for trypsin and chymotrypsin, enzyme and kernel protein were taken separately in 1 ml of 0.1 M potassium phosphate buffer (pH 7.6). Digestion was carried out at 37°C for 15 min and terminated by addition of 2 ml of 20% trichloroacetic acid (TCA). Absorbance at 280 nm of the TCA supernatant after centrifugation was read against reagent blank.

Cashew kernel protein extracted at pH 10.0 was denatured by heating for 10 min at different temperatures and *in vitro* digestibility by trypsin was studied. Denaturation was also done using SDS by heating the protein extracted at pH 10.0 with SDS and β-mercaptoethanol (SDS 4% and β-mercaptoethanol 10% final concentrations) for 2 min in a boiling water bath. SDS-denatured protein was

Table 1 *In vitro digestibility of cashew kernel protein by trypsin*

	Digestibility*	
Incubation time (min)	Tr. No. 1 kernel protein	BSA
5	0.07	0.036
15	0.084	0.082
30	0.11	0.119
60	0.134	0.201
90	0.144	0.223
120	0.137	0.232

*Expressed as increase in absorbance at 280 nm of TCA supernatant (see text for details).

Table 2 *Comparison of in vitro digestibility of cashew kernel protein, haemoglobin and BSA*

	Digestibility (%)	
Protein	Trypsin	α-Chymotrypsin
Haemoglobin	100 (0.088)*	100 (0.094)*
BSA	100	73
Cashew kernel protein		
i) pH 10.0 extract	133	62
ii) pH 4.0 extract	37	14

*Figures within parentheses are actual increase in absorbance at 280 nm of TCA supernatant. *In vitro* digestibility of BSA and cashew kernel protein is expressed as a percentage, taking digestibility of haemoglobin as 100. Kernel protein from cashew variety H-3-17 was used. Values are mean of three estimations.

Varietal variation

Table 3 *In vitro digestibility of kernel protein from different cashew varieties*

Variety	Trypsin		α-Chymotrypsin		Pepsin	
	pH 10.0	pH 4.0	pH 10.0	pH 4.0	pH 10.0	pH 4.0
H-3-17	100 (0.1133)*	100 (0.0367)*	100 (0.0515)*	100 (0.0207)*	100 (0.0373)*	100 (0.0583)*
Ansur-1	52	98	46	110	46	86
Vetore-56	42	136	44	72	131	39
WBDC-V	62	180	30	23	36	127
Morgaon-1	59	147	74	29	56	53
M 10/4	51	169	73	97	131	88
M 44/3	66	121	87	188	117	117
M 76/1	71	118	52	270	134	77
Tr No. 1	69	202	35	184	52	111
Tr No. 40	61	157	83	30	118	120
Tr No. 56	75	128	46	39	88	83
Tr No. 273	74	139	91	35	88	108
Bla-139-1	68	112	35	158	134	118
Bla-256-1	59	124	83	52	177	46
Bla-266-1	63	155	61	55	86	48
Vengurla-1	68	232	97	42	138	60
Vengurla-2	56	141	53	174	103	85
Vengurla-3	42	121	75	135	105	105
Vengurla-4	51	99	85	372	90	62
Vengurla-5	38	95	65	162	242	75

*See footnote to table 2.

used for digestibility studies after dialysis against 0.1 M potassium phosphate buffer (pH 7.6).

In vitro digestion by trypsin of cashew kernel protein from variety Tr. No. 1 extracted at pH 10.0 was carried out for different periods. The results are given in table 1. In the case of BSA the rate of reaction was found to be linear up to 60 min. However, in the case of cashew kernel protein, the rate of reaction was higher in the first 15 min. Hence, further studies were restricted to 15 min incubation.

A comparison of *in vitro* digestibility by trypsin and α-chymotrypsin of cashew kernel protein from variety H-3-17, and haemoglobin and BSA is shown in table 2. The digestibility of kernel protein extracted at pH 10.0 compared well with that of haemoglobin and BSA. However, the digestibility of kernel protein extracted at pH 4.0 was much less compared to that of BSA and haemoglobin.

Different cashew varieties differed in the *in vitro* digestibility of kernel protein (table 3). Protein extracted at pH 4.0 was less susceptible to digestion by trypsin and α-chymotrypsin compared to protein extracted at pH 10.0. Digestibility by pepsin was, however, slightly higher in pH 4.0-extracted protein than in pH 10.0-extracted protein.

In vitro digestibility by trypsin of denatured cashew kernel protein extracted at pH 10.0 increased with increase in denaturation temperature. Digestibility was maximum at 90°C. *In vitro* digestibility of heat- (90°C) and SDS-denatured cashew kernel

Table 4 *In vitro digestibility of denatured kernel protein from different cashew varieties*

Variety	Digestibility denatured (% increase over control)		
	Control*	Heat	SDS
Ansur-1	0.07	76	21
Vetore-56	0.073	103	0
WBDC-V	0.025	150	272
Morgaon-1	0.066	125	19
M 44/3	0.084	66	0
M 76/1	0.046	90	0
M 10/4	0.077	93	0
Tr No. 1	0.051	102	14
Tr No. 40	0.064	77	0
Tr No. 56	0.049	106	62
Tr No. 273	0.047	119	106
Bla-139-1	0.063	70	2
Bla-256-1	0.046	62	134
Bla-266-1	0.066	73	19
H-3-17	0.05	87	86
Vengurla-1	0.053	68	2
Vengurla-2	0.04	25	104
Vengurla-3	0.062	40	0
Vengurla-4	0.059	90	16
Vengurla-5	0.051	83	35

*Figures are actual increase in absorbance at 280 nm of TCA supernatant.

protein is shown in table 4. Both heat denaturation and SDS denaturation enhanced digestibility. However, SDS did not enhance digestibility in all the varieties. The varieties also showed significant differences in digestibility of heat-denatured kernel protein.

The results presented here clearly indicate that cashew varieties differ considerably in *in vitro* digestibility of kernel protein. Lysine content of kernel protein of these varieties has been shown to vary significantly[5]. Cashew kernel meal has been shown to contain proteinase inhibitors[8]. The observed differences in the *in vitro* digestibility of kernel protein among these varieties may possibly be due to variation in the total amino acid composition or variation in levels of proteinase inhibitors.

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ANNOUNCEMENT

SEMINARS ON INDUSTRIAL USES OF LEAD

Indian Lead Zinc Information Centre, New Delhi, and the Metallurgical Engineering Association (MEA), Department of Metallurgical Engineering, Indian Institute of Technology, Bombay, are jointly organizing the following three seminars at the Conference Hall, IIT Guest House, IIT, Powai, Bombay 400 076.

1) Lead Sheet for Corrosion Protection and Corrosive Fluid Handling in Chemical Industries—Saturday, 22 July 1989
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The registration fees for *each* of the seminars will be: Individual (per delegate), Rs 400/-; Company (maximum of 4 delegates), Rs 1500/-; Govt., educational and R & D institutions, Rs 200/-. Further details can be had from: Dr G. Sivaramaiah, Senior Technical Manager, Indian Lead Zinc Information Centre, No. 7, Shopping Centre, Block B-6, Safdarjung Enclave, New Delhi 110 029 (Phones: 600973, 604230, Grams: PLUMBOZINK, New Delhi 110 029, Telex: 31 72050 LZIC IN).

THE ADYAR BANYAN

A sense of dismay at its fall and a ray of hope at its replanting accompanied the recent events in the life of the Adyar Banyan Tree. The vast spread of this tree in the Theosophical Society campus at Madras had been accepted as a permanent feature by many who sheltered under its many-pillared canopy. The strong June winds sheared a part of the canopy and probably also the centuries-old main trunk. The ability of the remaining portions to let down new roots could in time fill the gap and, if the replanting effort succeeds, merge with the resurrected portion.

The banyan *Ficus benghalensis* L. belongs to the family Moraceae. The genus is paleotropical, with over 800 species. George King monographed the Indian representatives in 1888. Corner studied over 300 species in the living state and around 470 in herbarium collections.

Ficus benghalensis is a lowland tree extending to regions of drought and slight frost, where it becomes deciduous. It is recognized by its massive pillar roots and slightly cordate, softly hairy leaves with about 7 veins arising at the base of the lamina.

The round, sessile figs of the banyan turn red when ripe. They have a fascinating symbiotic relationship with insects, especially of the genus *Blastophaga.* The fig (syconium) is a fleshy receptacle, which by involution becomes hollow, leaving only a terminal pore covered by scales. There are numerous, tiny flowers on its inner surface. In the banyan the flowers near the pore (ostiole) are male while those away from the ostiole are female or sterile. The sterile flowers, known as gall-flowers, afford a place for the visiting insect to lay its eggs. In the egg-laying process, the pollen-dusted insect comes into contact with the stigma of a fertile female flower and pollinates it. Having completed its dual role, the insect flies away, dusted with fresh pollen from the male flowers in this receptacle.

The banyan is a symbol of hospitality—both in its many-pillared splendour and in its fruit-bearing. It is also a symbol of life. As Corner put it 'there seems to be no reason why it should die'.

Corner, E. J. H., Moraceae, in Dassanayake, *Revised Handbk. Fl. Ceylon*, 1981, p. 252.

King, G., The species of *Ficus* of the Indo-Malayan and Chinese countries, *Ann. Roy. Bot. Gard. Calcutta*, vol. I, 1888.

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BOOK REVIEWS

Annual Review of Entomology, Vol. 34, 1989, pp. 572, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, CA 94303–0897, USA), Price: US$38.

Another delightful volume of the *Annual Review of Entomology* has appeared, with 24 reviews on a variety of topics, from the bionomics of specific insects or pest groups to theoretical/evolutionary aspects, and, of course, the usual dose of extraordinary accuracy of technical details.

The ecology of *Heliothis*, a notorious pest of worldwide significance, is analysed by Fitt, who considers several of its biological dimensions and in the process highlights the relevance of wild, non-crop plants that invariably act as reservoirs, particularly during crop off-seasons. Continuing the discussion on this noctuid, King and Coleman illustrate the potential for controlling this insect using biological agents—an impressive list of entomophagous arthropods and microbes. This review contains useful information on the conservation, propagation and release of these natural enemies of *Heliothis*.

Appreciating the role of CO_2 in the complex interactions between insects and plants as well as between insects and mammals in the natural environment, particularly those that enable several invertebrates to perform 'oriented responses' to CO_2 gradients, Nicolas and Sillans provide an interesting account of the effects of naturally occurring high CO_2 on host-seeking behaviour and CO_2-mediated interaction between plants and pollinators. The CO_2-sensitive structures on the antennae of hymenopterans, on the labial palps of lepidopterans, and on the maxillary palps of dipterans necessarily indicate the evolutionary specializations of these insect groups. Critically examining caste evolution, community organization, foraging polymorphism, search pattern and territoriality, Traniello describes the foraging strategies of ants; his discussion relating to age polyethism and development of foraging behaviour will throw open newer avenues in ant behaviour research. The article on the ecology and behaviour of *Nezara viridula* by Todd discusses several details that would ultimately interest the farmer; his recommendation of the use of trap crops as a control strategy for the management of *N. viridula* appears ecologically sound.

Papaj and Prokopy highlight the evolution of 'learning' in phytophagous insects. Since the host plant is a major component of the insect's habitat, they suggest that insect behaviour can be monitored and quantified effectively by the experimental manipulation of the plant. By assessing the impact of learning, they affirm that the dynamics and coevolution of plant–herbivore systems can be established. The lock-and-key hypothesis, proposed nearly a century and a half ago, purporting to explain species-specific genetalic morphology in terms of mechanical reproductive isolation, has fared poorly in several attempts to test it, but it remains unfalsified in a definitive sense. Shapiro and Porter argue that it is unfalsifiable globally either in its unrigorous pre-darwinian form or in its darwinized form. They predict that as it is falsified for more and more specific cases, its potential domain becomes more and more restricted.

Since its coining and use from 1967, the term guild in insect ecology has gone through many newer interpretations and conceptual extensions. Through an extensive synthetic analysis, Hawkins and MacMahon clarify the status and usage of this term. The review on the structure and the function of deutocerebrum in insects by Homberg *et al.* should provide extremely interesting reading material, particularly to behavioural biologists, since it is the deutocerebrum that processes antennal sensory information initially.

Crawley's analysis of the effects of plant population dynamics *vis-à-vis* insect herbivory will set new research trends in the study of insect–plant interaction. The history of *Vedalia* beetle importation into California by Caltagirone and Doutt is interesting, especially against the backdrop of the early human perception of biocontrol in the late nineteenth century. The *Vedalia* success story should encourage strengthening the concept of biological pest control. Carpenter bees, essentially of the genus *Xylocopa*, are excellent natural pollinators of several horticultural plants. Discussing the bionomics of these bees, Gerling *et al.* offer a comprehensive overview of their seasonal activities, life cycles, nest types, nesting activities, floral associations, natural enemies, mutualism, mating behaviour, chemical communication, exocrinology, and origins and forms of sociality.

Maeda's review on the expression of foreign genes in insects using baculovirus vectors, and Meeusen and Warren's article on insect control on genetically engineered crops provide great insight into the biotechnological approach in modern entomological research. Soderlund and Bloomquist explain the neurotoxic actions of pyrethroids. They suggest that the voltage-sensitive sodium channel is the principal molecular target site for all pyrethroids, although to some extent, a voltage-sensitive calcium channel could also be involved. Entomological remote sensing using radar and special optical techniques has made major contributions to the study of insect flight, and in this context, Riley attempts to provide a detailed analysis of the processes involved, such as photography and videography from the air, multispectral scanning, radar and acoustic sounding. In the article 'Leafhopper and planthopper transmission of plant viruses', Nault and Ammar highlight the current trends in vector research involving new technologies, particularly the more sensitive serological methods and molecular probes.

The reviews on the ecology of the anthomyiid, *Delia*, in relation to vegetable crops, with adequate support information on its biological control, by Finch, the biology, host relations, and epidemiology of the scabies causer *Sarcoptes scabiei* by Arlian, the entomology of the oilseed *Brassica* by Lamb, the economics of pesticide resistance in arthropods by Knight and Norton, the bionomics of the Nabidae by Lattin, the chemical ecology and behavioural aspects of mosquito oviposition by Bentley and Day, and the enhanced biodegradation of insecticides in farm soil by Felsot provide extremely useful information.

To me, personally, reading this volume has been a good learning experience. The Editorial Committee has dedicated this volume to the Entomological Society of America which is celebrating its centennial this year. The ESA has indeed received a very rich tribute worthy of its name and traditions.

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Annual Review of Microbiology, Vol. 42, 1988, pp. 794, (Published by Annual Reviews Inc., 4139 El Camino Way, PO Box 10139, Palo Alto, California 94303, USA), Price: USA, $34; elsewhere, $38.

This volume has 29 articles beginning with an autobiographical sketch by R. G. E. Murray (University of Western Ontario, London, Ontario, Canada). There are 11 reviews on medical microbiology, 10 on microbial physiology and biochemistry, and 7 on microbial genetics and recombinant DNA technology. Most of the 29 reviews are from North American research institutions; 7 are exclusively from the UK, France or West Germany. The reviews are not only of current interest but are also exhaustive in their coverage.

Medical microbiology

D. A. J. Tyrell (Harvard Hospital, Salisbury, UK) describes clinical trials and mechanism of action of some new anti-viral compounds against the common cold virus. R. W. Emmons (California State Department of Health Services, Berkeley) reviews the ecology of the virus that causes Colorado tick fever. Shigellosis or bacillary dysentery caused by *Shigella* spp. is a result of poor sanitation in developing countries. The genetic determinants of *Shigella* pathogenicity have been outlined by A. T. Maurelli (Uniformed Services University of Health Sciences, Bethesda, USA) and P. J. Sansonetti (Institut Pasteur, Paris). Pathogenic bacteria and parasites successfully evade destruction mediated by the opsonic, chemotactic and lytic components of the complement system. K. A. Joiner (NIAID, National Institutes of Health, Bethesda), provides an overview of the subject and discusses strategies involved in the defence mechanism, citing the specific examples of *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Salmonella* spp. and other infections. *Aeromonas* and *Plesiomonas* have been recognized as pathogens of amphibians, reptiles, fish, snails, cows and humans. Sporadic gastrointestinal infections, osteomyelitis, septic arthritis, endocarditis, peritonitis, and eye and urinary infections have been attributed to *Aeromonas* spp. N. Khardori and V. Fainstein (University of Texas, Houston) describe the bacteriology and epidemiology of these unusual infections. Human skin affords a substrate congenial to the growth of bacteria and fungi which, in spite of the defense mechanisms, gain access to the body and cause infections. The ecological factors governing the colonization of these micro-organisms on skin have been elucidated by R. R. Roth and W. D. James (Walter Reed Army Medical Centre, Washington, DC). Aspergillosis, zygomycosis, candidiasis and trichosporonosis are some hospital-acquired fungal infections described in detail by T. J. Walsh and P. A. Pizzo (National Cancer Institute, Bethesda). The classification, isolation and properties of the simian immunodeficiency viruses have been described by R. C. Desrosiers (Harvard Medical School, Southborough, Massachusetts, USA). An understanding of the simian viruses may provide insight into the origin of viruses that cause AIDS in humans. *Actinobacillus*, *Bacteroides*, *Capnocytophaga*, *Fusobacterium*, *Rothia*, *Streptococcus* and *Veillonella* are genera of oral bacteria, which coaggregate and form the primary constituent of dental plaque. The ecological aspects of these bacteria have been studied and presented by P. E. Kolenbrander (National Institute of Dental Research, Bethesda). The prospects for vaccine development against filariasis caused by *Wuchereria*, *Brugia* and *Onchocerca* form the subject matter of an article by M. Philipp, T. B. Davis, N. Storey and C. K. S. Carlow (New England Biolabs Inc., Beverly, Massachusetts, USA). The Institut Pasteur in Paris is well known for research on medical problems. In a review of the contributions of this Institute M. Girard of the Institute relates the research into and development of viral vaccines for rabies, yellow fever, poliomyelitis, influenza and hepatitis B.

Physiology and biochemistry of micro-organisms

Bacillus subtilis is the most studied organism among the bacilli; the genetic systems involved in carbohydrate metabolism in *Bacillus* have been reviewed by A. F. Klier and G. Rapoport (Institut Pasteur, Paris). Bacteria offer simple biological systems for studying protein phosphorylation. The review by A. J. Cozzone (Université de Lyon, Villeurbanne, France) summarizes the biological role of bacteria in phosphorylation. Bioluminescent bacteria are classified into the genera *Vibrio*, *Photobacterium*, *Alteromonas* and *Xenorhabdus*. These bacteria emit blue-green light produced by a reaction catalysed by the enzyme luciferase. The bioluminescence operons of a number of marine bacteria have been cloned and the genes identified. An in-depth review of this aspect has been provided by E. A. Meighen (McGill University, Montreal, Canada). Species of the bacterial genera *Alcaligenes*, *Achromobacter*, *Pseudomonas* and *Rhodobacter* grow anaerobically and reduce ionic nitrogenous oxides to gaseous products. The review by L. I. Hochstein (Ames Research Centre, Moffett Field, California, USA) and G. A. Tomlinson (Santa Clara University, Santa Clara, California, USA) deals with the enzymes concerned with denitrification. The write-up by W. Reineke (Bergische University, Wuppertal, FRG) and H. -J. Knackmuss (Stuttgart University, Stuttgart, FRG) deals with the pathways for degradation of polychlorinated biphenyls (PCBs) and chlorinated benzenes, which reach the environment as pollutants. Progress in the area of anaerobic degradation of aromatic acids, phenols and hydrocarbons has been reviewed by W. C. Evans (University College of North Wales, Gwynedd, UK) and G. Fuchs (Ulm University, Ulm, FRG). During the first 20–40 min of the germination of spores of *Bacillus* spp., up to 40% of the total dormant spore protein gets degraded to free amino acids. The review by P. Setlow (University of Connecticut Health Centre, Farmington, USA) concentrates on this aspect and outlines some unanswered questions and future directions for research. The special sieving properties of the outer membrane of Gram-negative bacteria are due to the presence of a few major proteins known as porins. The structure and function of porins are the mainstay of an article by R. Benz (Wurzburg University, Wurzburg, FRG). Several protozoa lack mitochondria, and the peculiar metabolic properties of these organisms have been reviewed by M. Muller (Rockefeller University, New York). The precise factors that determine the site of virus assembly at cellular membranes are not well understood, and the review by E. B. Stephens (University of Florida, Gainesville, USA) and R. W. Compans (University of Alabama, Birmingham, USA) deals with the several unanswered questions about the mechanisms.

Microbial genetics and recombinant DNA technology

Baculoviruses—over 400 of them—have been isolated from invertebrates, and of these only 20 have been studied at the molecular level. A review by L. K. Miller (University of Georgia, Athens, USA) deals with recombinant virus production and protein synthesis using recombinant viruses. Kinetoplast DNA is the mitochondrial DNA of parasitic protozoa. The replication mechanism of this DNA in trypanosomes has been discussed by K. A. Ryan and associates (Johns Hopkins University School of Medicine, Baltimore). The current understanding of the genetics and biochemistry of *Streptomyces fradiae* and tylosin synthesis has been summarized by R. H. Baltz and E. T. Seno (Eli Lilly and Co., Indianapolis, USA). A detailed picture of the evolution of RNA viruses has been provided by J. H. Strauss and E. G. Strauss (California Institute of Technology, Pasadena). Resistance to arsenic and antimony is governed by plasmids, which also code for resistance to other heavy metals. Such plasmid-mediated resistance to heavy metals in bacteria has been reviewed by S. Silver and T. K. Misra (University of Illinois College of Medicine, Chicago). Certain basic information about the control of genetic transfer between *Agrobacterium tumefaciens* and the host plant serves as the basis of a review by A. N. Binns (Pennsylvania University, Philadelphia) and M. F. Thomashow (Michigan State University, East Lansing, USA). The genetic factors that enable a micro-organism to become a symbiont or a parasite in plants seems to be the basic theme of a discussion provided by N. T. Keen (University of California, Riverside) and B. Staskawicz (University of California, Berkeley). They cite the example of *Rhizobium* (a symbiont) and *Agrobacterium* (a parasite), which belong to the same family but interact in very different ways with plants.

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With the recent reorganization of Current Science we are introducing a section called 'News from Delhi', which will have, among others, articles and discussions about our Ministries, Departments, Commissions, etc. that deal with science and technology and about bodies that advise Government on matters scientific. The deliberations and advice of these organizations often end in governmental decisions that profoundly affect science and scientists in India. It is therefore vital that the scientists, who are really at the receiving end and who in the final analysis contribute to the growth of science, should know what these bodies are, what they do, and how and why decisions are taken. It is not uncommon to read in the newspapers that strong differences exist between these organizations. The media also have a tendency to sensationalize these differences. The scientific community is often baffled and sometimes embarrassed by these reports of divergence of views and is not able to grasp what the real issues are. Hence taking the scientists into confidence is an all-important step in the process of building up good science in the country.

In this context it gives us great pleasure to publish an article that tells us about the Science Advisory Council to the Prime Minister (SAC-PM) and its activities—an 'insider's view', as the author, Dr P. J. Lavakare, the Secretary to the Council, states. We hope this will help foster real communication between policy makers, science executives and working scientists.

— *Editor*

SCIENCE ADVISORY COUNCIL TO THE PRIME MINISTER—A BRIEF OVERVIEW

P. J. LAVAKARE
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Introduction

As a unique experiment, in February 1986, the Prime Minister appointed a Council of independent science advisors with fairly broad terms of reference. The Council was to advise the Prime Minister on:

a) major issues facing science and technology today;
b) the health of science and technology in the country and the direction in which it should move; and
c) a perspective plan for 2001 AD.

The Council was also to look at specific problems with different scientific departments, policies, priorities for research and technology missions, etc.

Unlike earlier approaches to setting up advisory committees, in which they mainly consisted of Government representatives from scientific departments and some individual scientists belonging to academies and major research institutes, the Science Advisory Council to the Prime Minister (SAC-PM) consists of a wide spectrum of individuals, including representatives from multinational companies, a few specialists in their individual capacity, and a Chairman who is not part of the Government machinery. At the outset, this combination of people, most of whom had not even interacted with each other before, looked certainly odd and attracted subdued criticism from members of the well-established scientific community. After a period of three years of functioning, it appears, at least to some of us, that the diversity of the group and their non-attachment to Government machinery have proved to be a source of strength in the form of innovative approaches and bold recommendations, which have arisen, not out of any personal interests, but with a perspective and with the main objective of using science and technology for national development. The following sections describe briefly (no doubt an insider's view) some of the activities of the Council over the last three years since its inception. This overview, to a certain extent, is also

SAC-PM

self-critical, and the fact that it is openly published confirms the approach that the Council is adopting in its deliberations. The overview describes some of the Council's activities over the last three years and its strategy for the next year, at the end of which its present term would expire. Through such a review the Council expects that it will provide its members an opportunity to focus their deliberations, during the last year of its term, on some specific issues that have not yet been resolved in terms of concrete implementation of their recommendations over the last three years. It will also use this opportunity to take up issues that it has not had the time to discuss so far, though they form part of the Council's terms of reference.

The Council has always recognized that the very advisory nature of its functioning is an indication that not all aspects of its recommendations may be implemented, particularly when the recommendations would demand major change in the existing system and since the recommendations to the Government are from a body that is outside the existing organizational framework. This limitation has not deterred the Council from taking up major issues that may cut across not only all the sectors of science and technology, but also those that affect the socioeconomic development of the country in a major way. Its deliberations have also come up with major recommendations, some of which have been resisted sternly by the existing Government machinery. The Council has always based its deliberations and recommendations on the fact that its main function is to provide independent advice to the Prime Minister on issues that are either already being dealt with within the Government system or on issues that need to be introduced in the normal functioning within the Government framework. Through the deliberations of the Council the members have, on several occasions, met the Prime Minister in formal and informal sessions and these sessions have often resulted in a closer understanding of the functioning of the Council based on the advice given by the Prime Minister. The Empowered Group, specially set up for considering the recommendations of the Council and consisting of Secretaries to the Government of India and chaired by the Cabinet Secretary, has deliberated on several recommendations sent to the Prime Minister and through such deliberations several of the recommendations of the Council have been incorporated in the Government's functioning, as would be indicated in subsequent paragraphs.

In addition to the approach of giving advice on matters referred to it by the Prime Minister, the Council, on its own, has taken several initiatives, within the framework of its terms of reference, and brought to the attention of the Prime Minister major issues related to science, technology and development. These include highlighting the development of priority areas (e.g. robotics, minerals development, advanced materials, parallel computing, lasers, photonics, chemical industry, agriculture, fertilizers, etc.) and suggesting specific action plans for developing these areas in the national framework. These initiatives of the Council have been, by and large, appreciated and the members of the scientific community have come forth, in a major way, to assist the Council in its deliberations on those issues where a wider expertise from the scientific community was utilized in the form of working groups and expert panels. In all these recommendations the Council's focus has been to come up with specific action plans so that 'things would happen' in the Government framework rather than expound on the elaborate technical details. It may be worthwhile to briefly describe how the Council functions in order to arrive at its recommendations.

How the Council functions

In view of the fact that the Council's members come from different backgrounds and have not always been in a position to interact on a day-to-day basis through normal governmental committee structures, one of the salient principles of the Council has been to organize its meetings on a very regular basis, with advance planning and ensuring a high rate of attendance by the members. The Secretariat of the Council has always been kept on its toes so as to make advance plans for its meetings, prepare well-focused agenda, and do quick follow-up of actions on the recommendations made by the Council. The Council has maintained a steady frequency of roughly one meeting per month and, as a result, has had 38 meetings over the first three years of its functioning. The emphasis at these meetings has always been to keep an atmosphere of informality and brevity, but at the same time, a well-defined focus on the issues involved and the specific recommendations that could emerge. Individual members, assisted by members of the scientific community, have separately provided inputs in the form of working group reports on various issues discussed by the Council.

Right from the beginning, the Council has been concerned about interacting with a wider cross-section of the scientific community and getting independent views from different cross-sections of the society. It had planned to hold these meetings in different parts of the country, but in spite of its conscious efforts, it has not been able to avoid having Delhi as its venue for 21 of its 38 meetings. The other meetings have been held in institutions located in Bombay, Goa, Hyderabad, Bangalore, Madras and Calcutta. It has not yet had an opportunity to hold its meetings in the north-east region of the country, where the problems of development have a unique character. The Council is very conscious of this lacuna and is hoping to make up for this in planning its future meetings. It is also conscious of the fact that, while these meetings are held in different locations, it would have to give more time to members of the scientific community for informal discussions and to obtain inputs that may be of relevance to its deliberations. In addition to the meetings of its own, the Council has met the Prime Minister on several occasions. All the meetings with the Prime Minister have given a great sense of satisfaction to the members as well as to the Secretariat, because these meetings also maintained an informal and frank atmosphere of discussions based on specific agenda items and were not bogged down by procedural matters and with issues connected with formalities. The Council has always made it a point to prepare for meetings with the Prime Minister in great depth, and the scientific and technical spirit with which an issue is to be presented is well rehearsed and clear-cut responsibilities identified for making audio-visual presentations to the Prime Minister. This has avoided dependence on bulky documents, except when detailed technical reports on areas of expertise had to be formally presented. The meetings with the Prime Minister have often enabled the Council to quickly convert the guidance of the Prime Minister into specific implementation mechanisms. Particular mention could be made of the role played by the Council in identifying a *modus operandi* for the technology missions initiated by the Prime Minister. Initiation of programmes on superconductivity, parallel computing and minerals development, formulation of an approach to the perspective plan for 2001 AD, setting up of a National Science and Engineering Research Board, etc. are some other examples where the deliberations of the Council and its direct interaction with the Prime Minister resulted in specific action plans followed up by the Government. The Council is always anxious and very ambitious that action is indeed taken on many of its recommendations and it has highlighted this aspect of its functioning by the close follow-up of its recommendations not only with the Prime Minister's office but, where necessary, with concerned Ministries and with the Cabinet Secretariat. In spite of these efforts, it has always asked itself the question, 'How can the Council be more effective?'

Recommendations

In its deliberations so far, the Council has made 36 concrete recommendations to the Prime Minister, and a close follow-up is maintained by the Secretariat on the consideration of these recommendations by the Government through the work of the Empowered Group chaired by the Cabinet Secretary. The recommendations are always directly sent to the Prime Minister's office, which decides on those that are to be considered by the Empowered Group. The Secretariat of the Council, which is also the Secretariat for the Empowered Group, pursues these recommendations, but unfortunately, within the Government system and within its operational framework and limitations, coupled with the multiplicity of departments even within the S&T sector, it has not always been possible for the Empowered Group to arrive at quick decisions on the various recommendations. The Council has recognized this as an inherent price one has to pay for a democratic decision-making process, which has its own virtue in that it brings about involvement of various Government organizations. But one often notices a sense of disappointment in the members of the Council when they find that their recommendations are tossed around from one forum to another and the decisions delayed. Can one blame the Council for being over-anxious about implementation of its recommendations? Or should the Council's members compromise with the Government mechanism of decision-making?

Technical reports

One of the salient features of the Council's activities has been the publication of a series of technical reports on the various topics mentioned earlier. These reports were not intended to be state-of-the-art reports, but mainly to highlight the importance of emerging areas and to indicate steps that the Government should take to initiate national activities in these identified areas. There are 11 such technical reports and several of them have been circulated to various Government Departments and to members of the scientific community. The Council is fully aware of the fact that these technical reports cannot stand on their own as professional reports that would stand the test of time as reference documents over the years. While these reports have identified the importance of these areas at a time when we must initiate national programmes in these areas, the Council has also considered the possibility of seeing whether it can play a role in producing (or setting up a mechanism for producing) these reports at a professional and academic level more in the form of technical reviews and with greater involvement of experts as well as other members of the academic community. While there may be a merit in preparing such detailed technical reports, there is the inherent danger that by the time such technical reports are prepared and edited and formally documented, the time for action would already have elapsed. It is with this concern that the Council has satisfied itself with bringing out technical reports that may have faced the general criticism by members of the scientific community of not being truly professional.

The Council has also been concerned with the question of management of natural resources from the point of view of meeting the requirements of the society as well as maintaining a balanced environment. Management of resources at the State and district levels has been given major attention by the Council and a specific report brought out based on the experience of the Karnataka State Council for Science and Technology. The Council also encouraged a pilot project for testing the feasibility of underground storage of flood water through programmes of deliberate excessive pumping of selected underground aquifers undertaken by the Agriculture Production Commissioner of the Uttar Pradesh State Government.

Perspective plan for 2001

One of the terms of reference of the Council was that relating to the perspective plan for 2001 AD. The Council approached this issue in a rather bold and innovative way, much to the surprise and chagrin of the official planners, who perhaps questioned the competence of the Council in undertaking this exercise. The Council was undaunted by these initial reactions and proceeded with its efforts, in the words of the Chairman, 'by writing from their heart what they felt the role that science and technology could play in the process of national development'. It categorized planning processing into two broad areas: (a) science and technology in planning the development of socioeconomic sectors, and (b) planning for the S&T sectors themselves. The question of integrating S&T with economic planning and setting the relative priorities within the S&T sector itself was once again the focus of the Council's approach and it boldly concluded that the existing mechanisms are not able to fulfil this objective unless the various organizational structures dealing with S&T-related activities are integrated. It commented on the multiplicity of S&T organizations that exist today and proposed the setting up of an apex body in the form of a National Science and Technology Commission, which will cut across all inter-sectoral needs of S&T in the socioeconomic sectors as well. This proposal of the Council has obviously resulted in raised eyebrows from several quarters of planners and decision-makers. Whether the Government accepts the proposal of the Council has yet to be seen but the perspective plan document of the Council—now often referred to as the red book—, unlike the usual Government documents, has certainly reached a wide cross-section of the community. The Council benefitted from the national debates organized at four different parts of the country—a suggestion emanating from the Prime Minister himself—and has been able to incorporate various suggestions in its final document, which also includes (in the typical approach of the Council) a series of 'recommendations for action'.

Issues to be discussed

Having reviewed its work during the first three years, the Council has considered a few selected areas on which it would like to concentrate its deliberations and provide a possible thrust to its activities during the last year of its term. One of such areas relates to the establishment of a Technology Development Fund which had been one of the recommendations of the Technology Policy Implementation Committee (TPIC) set up in 1983. The Technology Development Fund, which is expected to provide a boost to indigenous technology development, has not yet been established and the question of its operation by the Ministry of Industrial Development and its linkages with scientific departments and industrial R&D activity is of concern to the Council. The Council also feels that the Technology Information Forecasting and Assessment Council (TIFAC), which has also been set up as a result of the deliberations of TPIC and where the Council had also given advice, has to be closely linked with the activities of the Technology Development Fund in view of the related areas connected with technology information, forecasting and assessment. While the TIFAC is being coordinated by the Department of Science and Technology, the Technology Development Fund is expected to be set up under the Ministry of Industrial Development, and the close linkage vital to the success of these schemes is not yet clear. The Council is particularly concerned about the fact that R&D efforts in the country over the coming decade have to be considerably enhanced in the academic sector, in the national laboratories, in the socioeconomic Ministries and also in industry. Special thrust may be given by the Council to this area.

In the area of technical education, the Council had made very major recommendations highlighting the 'crisis' in technical education as it exists today and had clearly indicated specific action programmes for inclusion in the Eighth Plan proposals. The members of the Council are greatly concerned with this state of affairs and it is likely that they will pursue this area further. Another crisis area is population, and the Council has raised the moot question, When will India's population growth start decelarating? The Council is of the view that the problem of population must be tackled in a 'mission mode'. The mid-term appraisal of the Seventh Five-Year Plan has alarmed the Council because it shows that the rate of our population growth continues unabated. It believes that one of the most effective means of population control is widespread education of women and that the population control mission is chiefly societal and the S&T inputs are relatively marginal.

The entire question of increasing food production and the relevant agricultural inputs required has not yet been given due attention by the Council. No doubt it has initiated certain studies raising major issues in policies connected with fertilizer production and increased food production for the year 2001 AD. What implications these studies will have in terms of bringing about changes in the national programmes has yet to be clearly delineated. The Council is conscious of the fact that agriculture has to be given very high priority in the coming years and may come up with some very clear-cut recommendations in the coming months. The Council has also informally discussed the question connected with the vexing issue of brain drain, but since several tomes have already been written on this subject by expert groups and committees, the Council is cautious in its approach to this subject before it makes its own recommendations. One of the issues to which the Council may like to give its attention is the entire question of autonomy to be given to scientific research institutions and organizations. While the erstwhile Science Advisory Committee to the Cabinet (SACC) had deliberated on this issue in great detail and the Government has even come up with office orders 'giving' autonomy to scientific research organizations such as ICAR and ICMR, the Council is concerned with the fact that autonomy has not yet been introduced and the scientific community working in these institutions is still waiting to imbibe the spirit with which the erstwhile SACC had tackled this issue.

Highlighting the role of S&T in national development problems, the Council had strongly recommended that the State S&T Councils, set up in all States, must be fully activated and strong linkages forged between the Planning Commission and the various Central S&T agencies. According to the Council, the State Councils are expected to play a more active role in promoting competitive high-technology efforts, especially in the small-scale sector. The Council may continue its efforts to strengthen the role of State S&T Councils in the coming years.

It appears that the agenda of issues to be tackled by the Council is indeed vast and to make a major dent in the coming year in some of these issues would require a great deal of innovative thinking and bold decision-making. The question is: how will the Government respond to this call of the scientific community reflected through the recommendations of the Science Advisory Council to the Prime Minister?

Acknowledgement

The author is grateful to the Chairman and members of the Council for encouraging him to frankly write about some of the issues connected with the functioning of the Council. The views expressed are those of the author, but the Council does share the concerns expressed in the article.

CORRESPONDENCE

Science and audit

First I should like to congratulate the Editor for taking an innovative step by opening a page for discussion of issues that affect the doing of science *satisfactorily* and *effectively*. The financial aspects of supporting science (*Curr. Sci.*, **58**, 673) are one such issue; and given the present disposition of finance departments *vis-à-vis* scientists, it is only natural that much heat is generated when such issues are discussed. But what we need is the generation of light.

While Satyamurthy's statements may injure the feelings of some scientists, Venkataraman's over-protective reaction does not provide any soothing effect. We must therefore objectively analyse the major statements of Satyamurthy.

Satyamurthy says that huge sums of money are being spent now, compared to some twenty years ago, on science and technology. In absolute numbers, yes; but are we really spending huge amounts considering the declining purchasing power of the rupee, the tremendous growth in population, etc.? If we are truly committed to the goal of realizing social transformation and better living conditions through science and technology, then no amount is too big and no return is too insignificant. There is some truth in the statements made by Satyamurthy. For example, there is proliferation (to almost undesirable levels in some cases) in the cultivation of science and technology, like in many other fields. There is also lack of co-ordination among the various science and related departments of our government. In this, and in many other areas of science cultivation, the academies of science have a role cut out for them. But the point is: are they willing to shoulder the responsibility? I do not think scientists in India are profligate, as Satyamurthy would like the public to believe. On the contrary, the scientific community has, by and large, not been able to imbibe from other sectors of society the dubious virtue of being profligate. I can agree with Satyamurthy if he modifies his statement to say that there is lack of commitment to use monies effectively by some scientists or scientific organizations; but I cannot agree when he says that money is no problem for science. It is a big problem, especially for conscientious scientists who are not 'operators'. See the way our university-based scientists are languishing. Satyamurthy refers to the give-money-and-do-not-ask-questions syndrome among scientists. I do not think there is even a grain of truth in this statement. The finance departments must learn to ask pertinent questions and not those of the harassing type. We only hear about *audit objection*. Why can't we hear about *audit co-operation* or *audit assistance*?

The finance departments must cultivate the habit of functioning as referees or reviewers (like those existing for judging scientific papers for publication in a journal) rather than as hangsmen. This attitude will help foster the much-needed endearing environment between the scientific and financial communities, even if the latter wish to have the so-called *control* called *audit*. The referee of a scientific paper takes pains to offer such criticism that would make the paper acceptable for publication; similarly auditors can and should offer constructive criticisms rather than funnel out audit objections.

I think in all fairness to Satyamurthy he should be invited, at some stage after scientists have expressed their views on this subject, to have his say in these columns, since it is not uncommon to hear statements that the press has misquoted someone.

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Mr Satyamurthy has been contacted about replying to all the reactions by scientists.

—Editor

The 'impossible experiments' of Martin Fleischmann and his school

Martin Fleischmann has a remarkable track record of discoveries that were the results of unconventional experiments. To understand the phenomenon of adsorption of molecules on metallic surfaces, Martin Fleischmann used many new techniques, including Raman spectroscopy. In

1974 Fleischmann *et al.*[1] were the first to report enhancement of the intensity of Raman scattering by molecules of pyridine adsorbed on an electrolytically roughened silver surface. There was first much scepticism about this report till it was shown[2,3] without doubt that the enhancement is 10^6 times that predicted from the scattering cross-sections for bulk pyridine. The phenomenon is now known as surface-enhanced Raman scattering (SERS). It has been reviewed by Fleischmann and Hill[4] and is the subject of a monograph[5] published in 1982.

In the early eighties Fleischmann and his students opened up yet another area in electrochemistry. They found that shrinking the size of electrodes to a few microns was accompanied by many unexpected advantages[6-9], e.g. (a) they do not pose problems of charging current, (b) they could be used to electrolyse gases[10]! Such electrodes have been used to construct a scanning electrochemical microscope[11] with nanometre resolution. This has ushered in the era of microelectrodes in electrochemistry.

Martin Fleischmann always had a flair for the unconventional, a spirit that he has transmitted to his students. The world of science is waiting for an explanation for another of his new 'impossible experiments' (with Stanley Pons)—whether the observations are really due to cold fusion.

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ANNOUNCEMENT

INSA—SPONSORSHIP AND FINANCIAL ASSISTANCE FOR HOLDING CONFERENCES/SEMINARS/SYMPOSIA IN INDIA DURING 1989–90

Proposals for holding Conferences/Symposia/Seminars/Workshops in India are invited by the Indian National Science Academy for sponsorship with partial financial support. The Academy normally provides Rs. 5000 to Rs. 10,000 for a national conference/workshop and Rs. 10,000 to Rs. 20,000 for an international conference. The annual type of conferences/symposia are not encouraged by the Academy.

The application form may be obtained from the Project Officer (Basic Sciences), Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002.

ARTICLES

SOME PHYSICOCHEMICAL STUDIES ON ORGANIC EUTECTICS AND 1:2 ADDITION COMPOUNDS: *p*-PHENYLENEDIAMINE–α-NAPHTHOL AND *p*-PHENYLENEDIAMINE–β-NAPHTHOL SYSTEMS

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ABSTRACT

Phase diagrams of *p*-phenylenediamine–α-naphthol and *p*-phenylenediamine–β-naphthol systems determined by thaw melt method show the formation of 1:2 addition compounds. The solidification behaviour of pure components, eutectics and addition compounds, studied by measuring the rate of movement of growth front in a capillary, suggests that crystallization data obey Hillig–Turnbull equation. Infrared spectral studies of pure components and addition compounds indicate weak interactions among the components forming the molecular complex.

INTRODUCTION

The solid state chemistry of eutectics is an interesting[1-3] topic in materials science and metallurgy. The chemistry of eutectics has been a field of active investigation[4,5] during the recent past because eutectics have unusual physical properties not normally shown by the parent components. Metallic eutectics constitute an interesting area of research in metallurgy and materials science. However, owing to their low transformation temperature, ease in purification and transparency, and because of the wider choice of materials, organic eutectics are more suitable for investigation and have attracted the attention of many research groups[6-11]. Further, experimental techniques for organic systems are simpler and more convenient compared to those adopted for metallic systems. Besides, the small density difference of the two components forming an organic eutectic minimize density-driven convection effects on solidification. Most of the studies[12,13] in the past were on simple eutectic types. There are only a limited number of cases in which two components form 1:1 addition compounds[14-16] with congruent melting points. The molecular compounds are of potential technical interest since they are quite common in metallurgical systems. To the best of our knowledge there are no reports on studies of 1:2 addition compounds of organic origin that are organic analogues of intermetallic compounds. With a view to throw some light on solidification mechanisms and nature of interactions among the components forming eutectics and addition compounds, the phase diagram, linear velocity of crystallization and infrared spectral behaviour of *p*-phenylenediamine–α-naphthol (PPD–AN) and PPD–β-naphthol (PPD–BN) systems were studied.

METHODS

Phase diagrams of PPD–AN and PPD–BN were determined by the thaw melt method[17]. The linear velocity of crystallization was studied by the technique described earlier[18,19] using a glass U-tube (internal diameter 0.5 cm) with a 15-cm-long flat portion. The infrared spectra of pure components, eutectics and addition compounds were recorded[20] in nujol in the 4000–600 cm^{-1} region on a Perkin–Elmer 783 infrared spectrophotometer.

RESULTS AND DISCUSSION

Solid–liquid equilibrium data for PPD–AN and PPD–BN are plotted in figures 1 and 2 respectively. In both cases, there is a maximum, surrounded by two eutectics, and a molecular complex of 1:2 stoichiometry with congruent melting is formed. In the case of PPD–AN the two eutectics E_1 and E_2 have 0.38 and 0.93 mole fractions of AN, whereas in

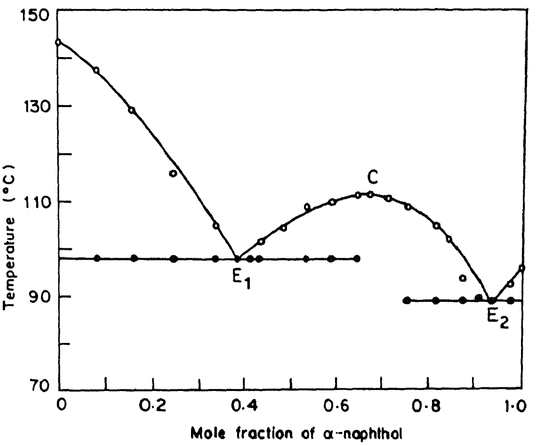

Figure 1. Phase diagram for PPD–AN. ○, Melting temperatures; ●, thaw temperatures.

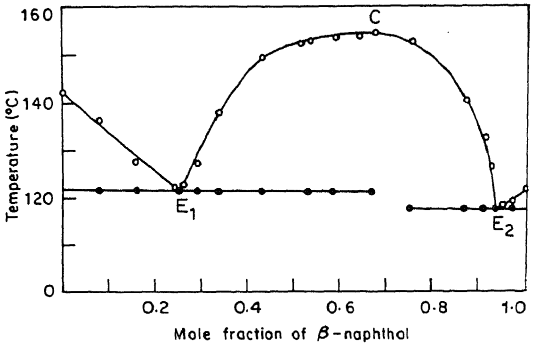

Figure 2. Phase diagram for PPD–BN. ○, Melting temperatures; ●, thaw temperatures.

PPD-BN the corresponding mole fractions of BN are 0.25 and 0.93. The eutectic temperatures for E_1 and E_2 in PPD–AN are 98.5 and 89.0°C respectively, and the corresponding eutectic temperatures in PPD–BN are 121.5 and 118.0°C. The melting points of the resulting addition compounds in PPD–AN and PPD–BN are 115.5 and 154.5°C respectively. From the phase diagrams it can be inferred that each system forms two eutectics and one 1:2 addition compound with congruent melting point indicated at C in the figures. For each eutectic, the addition compound serves as one of the components. The maximum in these systems is flat, indicating that the addition compounds are dissociated in the molten state. From the phase diagram it can also be inferred that these systems have addition compounds capable of existing as a solid in equilibrium with a liquid of the same composition.

Using the capillary method, the linear velocity of crystallization of the pure components, eutectics and 1:2 addition compounds was determined at different undercoolings. The experimental data plotted in the form of log v vs log ΔT are given in figures 3 and 4. The linearity of the logarithmic plots indicates the validity of the Hillig–Turnbull[21] relation

$$v = u(\Delta T)^n,$$

where u and n are constants and ΔT is undercooling. The experimental values of these constants are given in table 1. Various theories[17] have been developed for the growth mechanism of eutectics and predict a square relation between crystallization velocity and undercooling. The values of n in the present investigation are very close to two. The deviations[10] in values of n from 2, observed in some cases, may be due to the difference between bath temperature

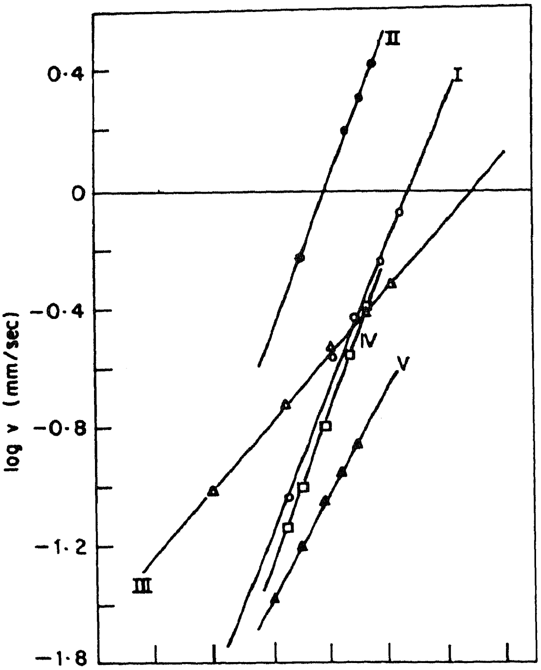

Figure 3. Linear velocity of crystallization at various degrees of undercooling for PPD–AN. [I, α-Naphthol; II, *p*-phenylenediamine; III, AN-PPD addition compound; IV, Eutectic 1; V, Eutectic 2.]

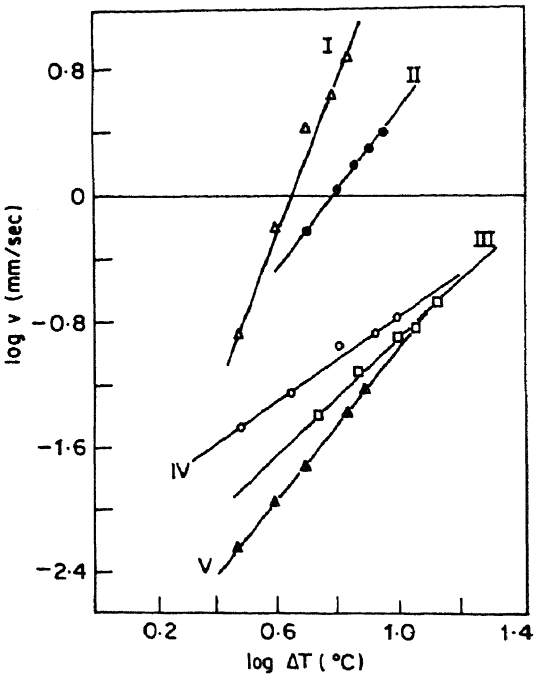

Figure 4. Linear velocity of crystallization at various degrees of undercooling for PPD–BN. [I, β-Naphthol; II, *p*-phenylenediamine; III, BN–PPD addition compound; IV, Eutectic 1; V, Eutectic 2.]

Table 1 *Values of u and n for PPD, AN, BN, and their eutectics and addition compounds*

System	*u* (mm/sec. deg)	*n*
PPD–AN		
PPD	0.01023	2.60
AN	0.00239	2.50
1:2 Addition compound	0.07243	1.14
Eutectic 1	0.00141	2.60
Eutectic 2	0.00763	1.80
PPD–BN		
BN	0.00092	4.66
1:2 Addition compound	0.00174	1.84
Eutectic 1	0.00945	1.33
Eutectic 2	0.00078	2.40

and temperature of growing interface. It is evident from figure 3 that the addition compound in PPD–AN crystallizes at a rate slower than that of PPD but faster than that of AN. From figure 4 it can be inferred that the crystallization rate of the PPD–BN addition compound is lower than the rates of both components. Studies on the crystal morphology of addition compounds indicate that they crystallize as a definite chemical entity. However, during crystallization the two components from the melt have to enter the crystal lattice simultaneously in such a way that the composition of the melt conforms to a 1:2 molar ratio of the two components. Because of this, the linear velocity of crystallization of the addition compounds may be expected to be of the order of the velocity of the species crystallizing at the slower rate.

It is evident from figure 3 that in PPD–AN the crystallization velocities of E_1 and E_2 are lower than those of the parent components and the 1:2 addition compound. E_1, with a low molar concentration of AN, solidifies faster than E_2, which has a high molar concentration of AN. The crystallization data for PPD–BN, given in figure 4, show that the crystallization rate of E_1 is lower than those of its parent components but higher than those of the addition compound and E_2. These results may be explained on the basis of the mechanism proposed by Winegard *et al*[22]. According to them eutectic solidification begins with the formation of the nucleus of one of the phases. This would grow until the surrounding liquid becomes rich in the other components and a stage is reached when the second component starts nucleating. Now there are two possibilities. First, the two initial crystals may grow side by side. This explains the cases in which the rates of solidification of eutectics are not lower than those of the parent components and addition compounds. The second possibility is that there may be alternate nucleation of the two components. This explains the solidification in the cases where the crystallization velocity of the eutectics is lower than those of the parent components and addition compounds. The solidification of the first eutectic E_1 of PPD–BN may take place by side-by-side growth of the two phases, whereas solidification of both eutectics of PPD–AN and the second eutectic E_2 of PPD–BN takes place by alternate nucleation of the two components. In these systems, the 1:2 addition compound behaves as one of the parent components for both the eutectics. The infrared spectrum of pure PPD has two weak characteristic bands, one at 3380 cm^{-1} and the other at 3300 cm^{-1}. The IR spectrum of AN gives a broad characteristic band[23] in the 3150–3350 cm^{-1} region. But the 1:2 addition compound of PPD–AN gives two strong bands, one at 3380 cm^{-1} and the other at 3300 cm^{-1}. The IR spectrum of pure BN also shows a broad characteristic band in the 3150–3350 cm^{-1} region. But the IR spectrum of the 1:2 addition compound of PPD–

BN gives two strong bands, one at 3380 cm^{-1} and the other at 3300 cm^{-1}. The increase in intensity[23] of 3380 cm^{-1} and 3300 cm^{-1} bands observed in the 1:2 addition compounds of AN and BN with PPD is due to intermolecular hydrogen bonding between the two components in each system.

ACKNOWLEDGEMENT

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5 August 1988; Revised 10 November 1988

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NEWS

S&T CO-OPERATION BETWEEN INDIA AND JAPAN

A joint meeting of the India–Japan Study Committee was held in New Delhi on March 2 and 3, 1989. The Indian delegation was headed by Sri Abid Hussain, Member, Planning Commission, and the Japanese team by Dr T. Mukaibo, Chairman, Atomic Energy Commission, Japan.

Some important recommendations of the India–Japan Study Committee include: (i) the establishment of a specific mechanism for taking up joint research projects in the fields of science and technology; (ii) increasing personnel exchanges between India and Japan, particularly involving scholars and scientists; (iii) the setting up of Indo–Japanese Information Centre to facilitate information exchange and development of contacts; and (iv) conducting joint studies in social sciences.

Current Science, July 20, 1989, Vol. 58, No. 14

EFFECT OF *CHLORELLA* DENSITY AND TEMPERATURE ON SOMATIC GROWTH AND AGE AT MATURITY OF THE ROTIFER *BRACHIONUS PATULUS* (MULLER) (ROTIFERA)

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ABSTRACT

The effects of three food levels (1, 2 and 4×10^6 cells/ml of *Chlorella*) and three temperatures (15, 25 and 35°C) on somatic growth and age at maturity of *Brachionus patulus* were investigated. Both growth rate and maximum body size reached were significantly higher at higher food levels. The effects of temperature alone on the adult body size did not show any clear trend. Higher food and temperature levels significantly reduced the age at maturity.

INTRODUCTION

Many morphological and life history parameters of rotifers, such as body size[1,2] and age at maturity[3], have been considered as products of natural selection and thus best adapted to their environment. However, the extent to which these parameters vary in rotifers in nature is often difficult to predict. For example, in *Keratella cochlearis*, when the morphometry was plotted as a function of altitude, marked differences appeared between the populations of the two different localities studied[4]. It is therefore only through experimentation that some conclusions can be drawn. In the present study the effects of food and temperature levels on the somatic growth and age at maturity of the rotifer *Brachionus patulus* (Muller) have been investigated.

MATERIALS AND METHODS

The general rotifer culture method was described earlier[5]. In the present study three food levels (1, 2 and 4×10^6 cells/ml of *Chlorella* sp.) and three temperatures (15, 25 and 35°C) were employed. For somatic growth studies 25-ml beakers were used as containers. Into each of 18 beakers (3 food levels × 3 temperatures × 2 replicates = 18) containing 20 ml of specified food density were introduced *B. patulus* neonates within 2 h of hatching at a density of 20 individuals/ml. The neonates were obtained as described earlier[6]. Test beakers were maintained in three temperature-controlled water baths set at 15, 25 and 35°C. At the start of the experiment and after every 12 h 10–15 individuals picked at random were removed from each test beaker and killed with dilute formalin. Morphometric measurements were determined as in earlier studies[6]. The experiment was discontinued when the individuals reached maturity, which was indicated by the appearance of ovigerous females.

To determine age at maturity (= age at first reproduction or AFR) 15-ml glass cavity blocks were used. The food density and temperature combinations used in the somatic study were also employed here. Into each of 27 cavity blocks (3 food levels × 3 temperatures × 3 replicates) 50 neonates were introduced. At the start and after every 2 ± 0.5 h interval each individual in each cavity block was examined and the ovigerous females were counted and removed. The non-ovigerous females were transferred to fresh medium of appropriate food density and temperature after every 12 h. At 15°C, as the time to attainment of maturity was very long (120 h), observations were made at 12 h intervals only.

RESULTS AND DISCUSSION

Many factors appear to influence body size of rotifers. These include genotype[7], predation[8,9], salinity[10] and food quality[11]. However, within a clone of rotifer species the most significant factors influencing body size are food density[6,12] and temperature[13,14]. Somatic growth in *B. patulus* occurred only till AFR. No measurable growth in body size (lorica area) occurred beyond AFR regardless of food density and temperature (figure 1).

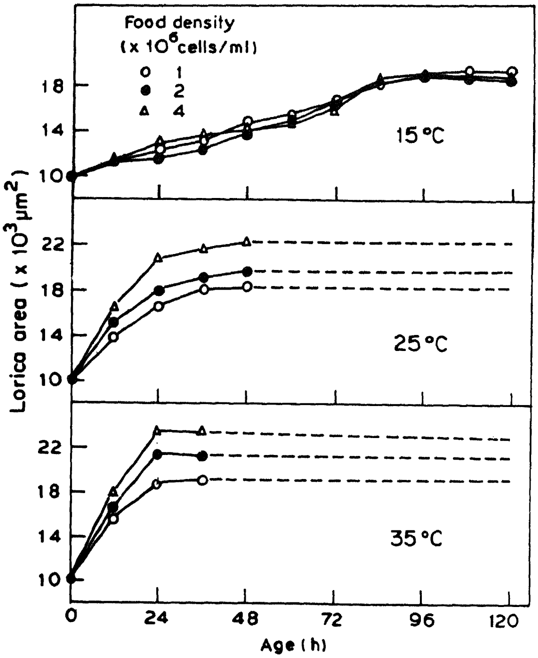

Figure 1. Somatic growth as increase in lorica area of *B. patulus* in relation to food density and temperature. Each point represents mean of 10–25 measurements from each of the two replicates. Broken lines indicate no measurable growth in lorica area.

The growth rate up to AFR was significantly influenced by both food density and temperature. The effect of food density–temperature interaction was also significant. Both growth rate and maximum body size reached were significantly higher at high food density and temperature. Significant differences in body size of rotifers grown at different food density–temperature combinations were evident as early as 12 h after hatching (table 1). The highest specific growth rate (maximum increase in lorica area/time (h) taken to reach that size) at 35°C and 4×10^6 cells/ml food density was nearly seven times higher than that achieved at 15°C at the same food level (figure 2).

The period from hatching to age at maturity is generally subjected to maximum environmental influence[9]. It is only during this period that somatic growth takes place because of active feeding[15]. Since all cell divisions are completed before hatching, the somatic growth of rotifers is mainly because of increase in cell size due to incorporation of assimilated material[16]. It is therefore reasonable to predict the development of larger individuals under optimal food density. The somatic growth rate in rotifers may be measured in terms of lorica area[6] or dry weight[17,18]. The measurement of lorica area as an index of rotifer size may be more reliable as it is independent of the quantity of food present in the

Table 1 *Effect of food density and temperature on somatic growth and age at maturity of B. patulus: Two-way analysis of variance*

Parameter	Source of variation	df	SS	MS	F
Specific growth rate	Food density	2	63724	31862	115.7**
	Temperature	2	475906	237953	864**
	Food density × temp. interaction	4	40644	10161	36.9**
	Error	9	2478	275	
Growth rate at 12 h	Food density	2	9.75	4.87	99.47**
	Temperature	2	96.80	48.40	987.96**
	Food density × temp. interaction	4	5.98	1.49	30.53**
	Error	9	0.44	0.04	
Growth rate at 36 h	Food density	2	23.68	11.84	95.48**
	Temperature	2	216.90	108.45	894.60**
	Food density × temp. interaction	4	10.04	2.51	20.24**
	Error	9	1.12	0.12	
Age at maturity	Food density	2	194	97	8.12*
	Temperature	2	48375	24188	2026**
	Food density × temp. interaction	4	79	19.75	1.65^{ns}
	Error	18	215	11.94	

**Highly significant $P < 0.001$; *Significant $P < 0.01$; ^{ns}Non-significant $P > 0.05$.

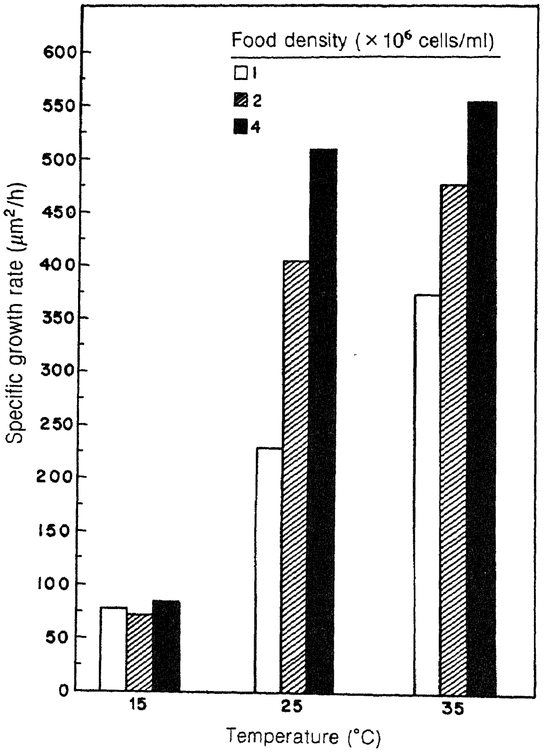

Figure 2. Specific growth rate (increase in lorica area/h) of *B. patulus* under different food density–temperature conditions. Values shown are based on 10–25 measurements from each of the two replicates.

gut. On the other hand, if rotifer body size is measured in terms of dry weight, the physiological state of the animals, such as nutritional status, stage of developing embryo within female body, etc., which are usually difficult to define, should be specified.

The growth patterns recorded in the present study are similar to those reported by others[16,19] in that the maximum possible growth was accomplished soon after hatching (except at 15°C) and increase in food level resulted in increased lorica area[20]. The effect of temperature alone on the adult body size was not clear from those results and probably prolonged exposure to a particular temperature may be needed[10]. Rotifers collected in winter had larger loricae than those collected in summer[21,22]. However, other factor such as predation[8,9] may also influence rotifer body size in nature. The increase in body size with increase in food density was evident even after age at maturity (figure 1). There is some evidence that excessive food results in decreased body size in rotifers. Pilarska[12] noticed that the largest juveniles and mature *B. rubens* were those grown at optimal food conditions; there was a general decrease in body size under both low and high food levels.

The differences in growth rates of rotifers kept under different test conditions were also reflected in age at maturity. In general both food level and temperature had significant effect on AFR (table 1). Higher food and temperature levels contributed to reduced AFR, i.e. maturity was reached earlier. The AFR was highest (5 d) at 15°C and low food level and smallest (0.9 d) at 35°C under high food level (figure 3). Reduction in AFR with increasing food availability, as observed in *B. patulus*, was reported earlier in two other rotifer species, *Asplanchna girodi*[23] and *Euchlanis dilatata*[16]. Similarly reduced AFR at higher temperature were also recorded in *Asplanchna brightwelli*[24], *B. calyciflorus*[25] and *B. dimidiatus*[26].

The food levels chosen in the present study were within the range used for other rotifers[27]. The

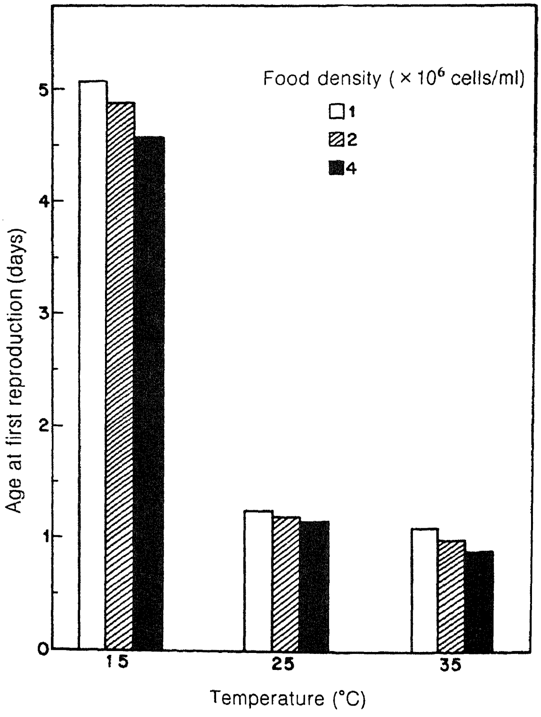

Figure 3. Influence of food density and temperature on age at maturity of *B. patulus*. Fifty neonates were employed for each food density–temperature combination.

temperatures employed here (15–35°C) are in the range that *B. patulus* experiences in nature[28]. However, temperatures higher than 35°C showed marked contrastive effects on rotifers. For example, in *B. calyciflorus* and *B. urceolaris*, increase of temperature to 40°C actually prolonged the juvenile phase, although in no regular manner[27].

In freshwater ecosystems rotifers in a variety of habitats are just as much successful as cladocerans and copepods. What copepods to a large extent and cladocerans to a certain extent achieve through their high fecundity, rotifers achieve through their quicker maturing (earlier age at first reproduction)[3]. The large seasonal and geographical variations in the dynamics of many rotifer species may be at least partly explained by the effects of food density and temperature on body size and age at maturity.

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SHORT COMMUNICATIONS

DIPOLE MOMENTS OF SOME ARYLMERCURIC CHLORIDES—VALENCE ANGLE OF MERCURY

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THE present study of the dipole moments of arylmercuric chlorides was undertaken (i) to determine, at least approximately, the valence angle that mercury assumes in these compounds, and (ii) to find out whether mercury can act as an electron acceptor conjugating with electron-releasing groups present in the *para* position of the aryl nucleus.

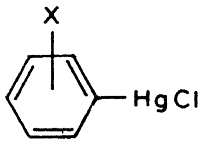
The arylmercuric chlorides were prepared according to methods described earlier[1–5]. 2,6-Dimethylphenylmercuric chloride was a new compound, m.p. 156–157°C. Found: C, 28.2; H, 2.5. $C_8H_{10}HgCl$ required: C, 28.2; H, 2.7%.

The apparatus used for the determination of dipole moments and the method of calculation have been described earlier[6]. All the measurements were made in dioxane solution (because of insolubility of the compounds in benzene) at 30±0.05°C. The dipole moments are given in table 1.

The angle that the dipole axis makes with the 1–4 axis in arylmercuric chlorides can be estimated from group moments by vector addition. According to the Fuchs'[7] equation

$$\mu^2 = \mu_1^2 + \mu_2^2 + 2\mu_1\mu_2 \cos\theta \cos\alpha \cos\beta, \quad (1)$$

in a compound like I,



I

μ is the dipole moment of the molecule, μ_1 is the moment of group X, μ_2 is the moment of –HgCl, θ is the angle between the axes of rotation of the groups, and α and β are the angles that the moments make with their respective axes of rotation. The axes of rotation are assumed to be directed to the group. Using (1) and the moments of *m*-chlorophenylmercuric chloride, and –Cl and –HgCl groups, the angle that the moment of –HgCl makes with its axis of rotation can be calculated. The moments of chlorobenzene[8] (1.61 D in dioxane at 30°C) and phenylmercuric chloride (3.11 D) may be taken as the moments of –Cl and –HgCl groups respectively. Computation of the angle that the dipole axis makes with the C–Hg bond gives a value of 7°. The moment angle can also be calculated using the moment of *p*-chlorophenylmercuric chloride, and this gives 18°. But we can exclude it from consideration in view of the possibility of conjugative interaction between *p*-Cl and –HgCl groups. Sipos *et al.*[4] calculated the angle to be 50° from their dipole moment of 2.91 D for *m*-chlorophenylmercuric chloride but we found, by repeated determinations using a highly purified sample, that the dipole moment of the *m*-chloro compound is only 2.70±0.02 D. The value of 2.91 D of Sipos *et al.*, therefore, seems to be in error and consequently the moment angle of 50° is also unacceptable.

There is a critical dependence of the moment angle on the dipole moment of arylmercuric chloride. For example, if the dipole moment of *m*-chlorophenylmercuric chloride is 0.01 D less (i.e. if taken as 2.69 D instead of as 2.70 D), the angle will be almost

Table 1 *Dipole moments of arylmercuric chlorides in dioxane at 30°C*

No.	Compound	Dipole moment (D)
1.	C_6H_5HgCl	3.11*
2.	*o*-$CH_3.C_6H_4.HgCl$	2.98
3.	*m*-$CH_3.C_6H_4.HgCl$	3.22
4.	*p*-$CH_3.C_6H_4.HgCl$	3.45
5.	2,6-$(CH_3)_2.C_6H_3.HgCl$	3.07
6.	*m*-$Cl.C_6H_4.HgCl$	2.70**
7.	*p*-$Cl.C_6H_4.HgCl$	1.65
8.	*o*-$HO.C_6H_4.HgCl$	4.02
9.	*p*-$(C_2H_5)_2N.C_6H_4.HgCl$	5.07
10.	*p*-$(CH_3)_2N.C_6H_4.HgCl$	4.94
11.	2-CH_3-4-$(CH_3)_2N:C_6H_3.HgCl$	4.87

*Lit[4]. value, 2.99 D; **Lit[4]. value, 2.91 D.

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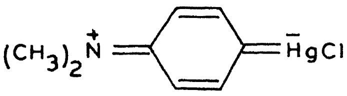
*For correspondence.

180°. In view of the fact that the observed dipole moment can vary by ± 0.02 D owing to experimental errors, the moment angles or bond angles computed from dipole moments are approximate and should only serve as useful guides in understanding the structure of molecules. Hence it may be concluded that the mercury valence angle in arylmercuric chlorides is very nearly 180°. This conclusion is also in accordance with the view that the two σ-bonds of mercury, resulting from 6*s*, 6*p* hybridized orbitals, should be linear.

The observed dipole moments 5.07 D and 4.94 D for the compounds 9 and 10 of table 1 are higher than the sums (4.92 D and 4.74 D respectively) of the group moments (dipole moments: $C_6H_5N(C_2H_5)_2$, 1.81 D[9]; $C_6H_5N(CH_3)_2$, 1.63 D[10]; C_6H_5HgCl, 3.11 D). The observed moment cannot be higher than the sum of the group moments without the existence of conjugative interaction of the *para* substituents. For example, the high moment of *p*-dimethylaminophenylmercuric chloride should be due to structure II making some contribution to its ground state.



II

This structure involves the utilization of one of its vacant 6*p* orbitals by mercury. Mercury can thus act as an electron acceptor and show a valence of 3.

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INTENSITY VARIATION OF X-RAY REFLECTIONS IN SINTERED MATERIALS

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SINTERED materials, with desirable mechanical, electrical and magnetic properties, are gradually replacing unsintered materials. Sintering is an agglomeration or consolidation of loosely packed or pressed fine powder particles by heat treatment below the melting temperature. Many attempts have been made to understand the phenomena involved in sintering. Metallic sintering was considered to be a diffusion process[1]. The initial stage of sintering[2] of solids by viscous flow, evaporation and condensation and self-diffusion was also suggested. Sintering was considered to occur by the rearrangement of particles at low temperatures in copper and silver powder[3,4]. Recently it has also been described as transport of mass[5]. But so far no satisfactory explanation of the mechanism of sintering has been available. It was felt that X-ray diffraction would be useful in studying the problem.

Samples were prepared from copper powder of size 100 μm obtained from Bharat Metallic Powders Limited, India. The copper powder was pressed in a stainless steel die of the push–pull type. The cylindrical powder pellets were 13.1 mm in diameter. Polyvinyl alcohol was used as binder as it is volatile at about 573 K. The pellets thus prepared were sintered by keeping them in granular petroleum coke powder at temperatures in the range 773 to 1273 K for five different durations of sintering.

X-ray diffraction studies of the sintered samples were done on a Philips P. W. 1730 X-ray diffractometer. In order to study the variation in intensity of diffracted X-rays as a function of temperature parameters like pressure, duration of sintering and the size of the samples were kept constant. Diffractometer parameters like radiation, filter, tube voltage, current, chart speed, scanning rate, time constant, etc were also kept constant. It can be observed from the curve of $I_{(111)}/I_{(200)}$ versus sintering temperature (figure 1) that there is a change in the intensity of reflections with temperature. The (hkl) reflections were found to vary in intensity systematically (table 1). A plot of intensity of X-ray reflections versus sintering temperature is shown in figure 2.

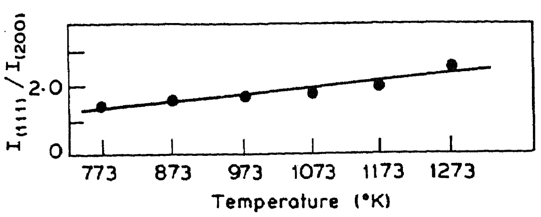

Figure 1. Variation of I_{111}/I_{200} with sintering temperature.

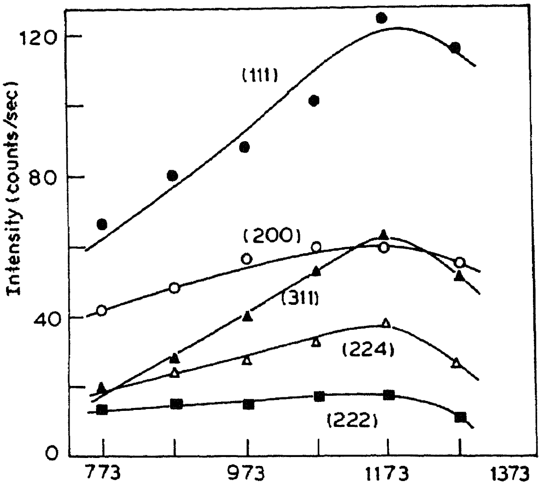

Figure 2. Effect of temperature on the X-ray intensities of different (hkl) reflections.

The intensity of X-ray reflections increases up to 1173 K and beyond this temperature decreases gradually. The increase in intensity was more for the (111), (311) and (220) planes and comparatively less in the case of the (200) and (222) planes. The increase in intensity with increasing sintering temperature indicates increase in density of the corresponding (hkl) planes of the grains in the compacts.

Three mechanisms have been proposed to contribute densification[5,6]. But Eadie and Weatherly[7] have observed the following for copper: (i) If the homologous temperature is <0.75 of the melting point and the neck radius is $<5\,\mu$m, then grain boundary diffusion dominates. (ii) If the homologous temperature is >0.95 of the melting point and the neck radius is $>20\,\mu$m, volume diffusion dominates. The confusion in the literature with regard to the mechanism of sintering was resolved by German and Munir[6] with reference to the work of Nyce and Shafer[8]: in copper grain boundary diffusion takes place around 1023 K while volume diffusion occurs for compacts of large particles maintained at higher temperatures, around 1123 K. Masuda and Watanabe[5] concluded that at lower temperatures the grain boundary diffusion contributes to smaller neck sizes and densification. With increase in sintering temperature, the volume diffusion contributes to larger neck size and greater densification. These facts are also reflected in our X-ray intensity observations.

At 773 K and 873 K surface diffusion favours the growth of the neck radius and grain boundary diffusion leads to densification, as revealed by the slight rise in intensity in that interval. Between 873 and 1173 K the appreciable rise in intensity implies increasing densification of the grains due to grain boundary diffusion alone because the surface flow decreases as the neck radius increases and stops finally beyond a critical value of the neck radius. At 1173 K volume diffusion has been confirmed for copper[9]. Thus grain boundary diffusion and volume diffusion contribute to the maximum densification, which is reflected in the maximum intensity at 1173 K in figure 2.

The decrease in intensity beyond 1173 K can be attributed partly to onset of recrystallization. According to Novikov *et al*[10] recrystallization is

Table 1 *Intensity of X-ray reflections from sintered samples made of 100 μm Cu powder at 600 MPa pressure after 4 h of sintering*

	Intensity of (hkl) reflections (counts/second)				
Temperature (K)	(111)	(200)	(220)	(311)	(222)
773	66	42	20	20	14
873	80	48	25	28	16
973	88	56	28	40	16
1073	100	59	34	52	18
1173	124	60	38	62	18
1273	116	56	28	52	12

also possible during sintering. It is accompanied by a kinetic phenomenon of increased excess vacancy concentration near the melting point. Thus negative shrinkage[11] is also a consequence of recrystallization. The decrease in intensity at 1273 K must be due to the vacancy concentration by recrystallization, which is favoured by the reduction in surface energy at temperatures a little below the melting point.

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A NEW APPARATUS FOR THE DETERMINATION OF GEL STRENGTH OF PHYCOCOLLOIDS

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THE quality of phycocolloids such as agar agar and carrageenan is usually determined by their gel strength[1,2]. The methods of Nikan Sui and Rower Bal for measuring gel strength are basic controls in the agar market, yet they are not mentioned in the Pharmacopoeias, National Formulary, Codex, or other similar publications or specifications and analytical methods referring to phycocolloids[2]. The principle involved in the determination of gel strength is based on measuring the load (g cm^{-2}) that causes a standard gel (1.5%) to break within a few seconds. In the present attempt a new design for the determination of gel strength has been presented.

A funnel with a basal area of 0.31 cm^2 is attached to a string which is passed over a single fixed pulley. The other end of the string is attached to a weight w. The initial weight of the funnel is p. The weight w should be equal to p. The funnel when initially made to rest on the gel can be considered to be of 'zero' weight as the initial weight p is neutralized by the weight w at the other end of the string (figure 1).

A hot 100 ml of 1.5% solution of phycocolloid was prepared with glass-distilled water and poured into a 500 ml beaker and kept for 24 h at room temperature. The gel was placed under the instrument and the funnel was allowed to rest over the gel. Lead shots were slowly added to the funnel without any disturbance. At a particular weight when the gel was broken, the lead shots were removed and weighed. This weight was divided by the basal area 0.31 cm^2 and expressed as gel strength in g cm^{-2}.

In any ideal machine, the efficiency is equal to unity.

$$\text{Efficiency} = \frac{\text{Mechanical advantage}}{\text{Velocity ratio.}}$$

In a simple machine, when the power p just balances the load w, the ratio of the load to the power is called the mechanical advantage, i.e. w/p. In this mechanism the mechanical advantage is unity as w should be equal to p initially.

Let the initial length of the string resting on the gel be L_1 and the length with weight w be L_2. After the breaking of the gel, the length of the string on

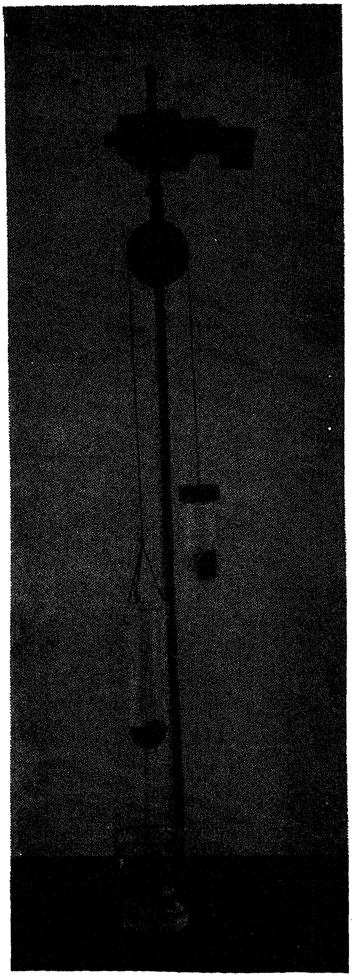

Figure 1. Set-up for gel strength measurement.

the gel is L_3 and the length of string with weight w is L_4. Now,

$$L_3 - L_1 = x$$
$$L_2 - L_4 = y.$$

Velocity ratio $= x/y$, i.e. velocity ratio is equal to unity as the increase in length x is equal to the decrease in length y.

Efficiency in this instrument is unity since

$$\text{Efficiency} = \frac{\text{Mechanical advantage}}{\text{Velocity ratio}} = 1/1 = 1.$$

The gel strength of agar sample at 1.5% concentration in glass-distilled water was determined by a Nikan Sui type instrument made by Kiya Seisakusho Ltd, Japan, at the Central Salt and Marine Chemicals Research Institute, Bhavnagar, and was found to be identical with that measured using the instrument devised by us.

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PHOTOCHROMIC ACTIVITY OF 4-HYDROXYBENZYLIDENESALICYLOYLHYDRAZINE

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VARIOUS types of organic compounds have been investigated for photochromic activity. Generally this phenomenon occurs owing to the structural isomerism with the effect of radiation[1,2]. Cohen *et al.*[3-5] considered that the presence of the *ortho* hydroxy group is an essential condition for coloration in *N*-salicylideneanilines and interpreted photochromism as an intrinsic property of the individual anil molecule. Benzenesulphonylhydrazines are photochromic in rigid matrices at low temperature[6]. Arylidene hydrazides, which are structurally similar to benzenesulphonylhydrazines, are potentially photochromic. An attempt was therefore, made to prepare arylidene hydrazides and investigate their photochromic activity. These hydrazine derivatives were prepared by condensation of the appropriate acid hydrazide (prepared by refluxing hydrazine

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*For correspondence.

UV radiation

hydrate with ester) with the corresponding aldehyde in ethanol heated over a water bath for 3 h[7]. Crystals obtained on cooling were further purified by recrystallization from petroleum ether–acetone. Purity was checked by TLC, elemental analysis, and IR and PMR studies.

4-Hydroxybenzylidenesalicyloylhydrazine (m.p. 231°C) showed the colour change in crystalline form from white to yellow on exposure to sunlight for 5 min and reversal to the original colour thermally in the dark or on keeping in the dark at 25°C for a week. The cycle could be repeated several times without any fatigue in the molecule. The compound did not show any colour change when exposed to UV light (365 nm). When either of the OH groups was replaced or substituted by the $-OCH_3$ group, the photochromic property of the compound was lost.

Photochromism among *N*-salicylideneanilines has been attributed to the existence of intramolecular hydrogen bonding between the phenolic proton and the imine nitrogen leading to intramolecular hydrogen transfer followed by *cis-trans* isomerization yielding photochromic coloured species on excitation[5]. Compounds with an *ortho* group other than OH have also shown photochromic behaviour[8,9], and the photochromism involves an intramolecular proton shift from a suitable *ortho* group to the nitrogen of the $>C=N$ leading to quinoid-type structure.

In the present case 2-hydroxybenzylidenesalicyloylhydrazine did not show observable colour change on irradiation (UV, sunlight) in the solid state whereas 4-hydroxybenzylidenesalicyloylhydrazine is photochromic in the solid state (sunlight). The observation, indicates that *ortho* groups that are able to shift their proton intramolecularly to the azomethine nitrogen are not essential for photochromism in this series of compounds. Further investigations of the mechanism of photochromism in this series of compounds are under progress.

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EXTRACTION AND SPECTROPHOTOMETRIC DETERMINATION OF IRON IN STEELS AND HAEMATITE ORE USING THIOCYANATE AND CHLORPROMAZINE

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MANY methods have been reported for the spectrophotometric determination of iron in steel, ores, rocks and minerals[1]. However, these methods are not well suited for simple and selective determination of iron because of complicated procedures or chemical interferences. The thiocyanate method is applicable in routine analysis, but it has serious shortcomings such as the formation of more than one product with iron(III), $[Fe(SCN)_n]^{+3-n}$, $n = 1 \ldots. 6$, depending on the concentration of thiocyanate. In the work described here chlorpromazine (CPZ) was chosen as a reagent to establish a new, simple, rapid and selective extraction and spectrophotometric determination of microamounts of iron in haematite and steels.

A Beckman Model DB, UV–VIS spectrophotometer with 1 cm matched silica cells was used for absorbance measurements. Stock solution of iron(III) was prepared by dissolving iron(III) ammonium sulphate (AnalaR, BDH) in double-distilled water with a few drops of sulphuric acid. This stock solution was standardized gravimetrically[2] and used after appropriate dilutions as and when required. 0.05 M CPZ solution in chloroform, and ammonium thiocyanate, 25% w/v in water, were also prepared.

Various aliquots of iron solution containing

1–27.5 μg of metal were transferred into a series of separatory funnels. Two ml of 10 M H_2SO_4 and 5 ml of 25% ammonium thiocyanate were added and the solutions were diluted to 20 ml with distilled water. Ten ml of 0.05 M CPZ in chloroform was added and the mixture was equilibrated. The chloroform layer was collected in a 10-ml standard flask and the absorbance of each extract at 480 nm was measured against a similarly prepared reagent blank. A calibration graph was drawn and was subsequently used in sample determination.

CPZ reacts with iron(III)–thiocyanate in acidic medium to form a yellow ion-association complex which can be extracted into chloroform. This extract shows λ_{max} of 480 nm. A study of the possibility of extraction of the ion-association complex from hydrochloric, sulphuric, acetic, perchloric and nitric acid media was carried out. There was no complex formation in nitric, acetic and perchloric acid media. Sulphuric acid and hydrochloric acid were found to be suitable for the formation and extraction of the ion-association complex, offering rapidity, sensitivity and stability of the colour reaction between iron and thiocyanate. A 0.1–1.2 M HCl or 0.2–2.5 M H_2SO_4 concentration was found to be essential for getting a clear yellow extract of the complex. Below this level of acidity the organic extract becomes turbid after a few minutes of extraction.

The effects of the amounts of reagents added on the extraction of iron were studied. The results indicated that for between 2 and 10 ml of 25% ammonium thiocyanate the absorbance of the extract had a constant maximum absorbance at 480 nm. Similarly, for 5 to 10 ml of 0.5% CPZ in chloroform, the organic extract showed constant absorbance.

An equilibration time of 1 to 2 min was sufficient for maximum recovery of iron into chloroform. Shaking the mixture for up to 10 min did not alter the colour intensity of the complex. Changing the volume of the aqueous phase from 10 ml to 50 ml did not affect the absorbance of the organic extract. Of the various solvents, viz. benzene, toluene, chloroform, dichloromethane and carbon tetrachloride, investigated for extraction of the iron-association complex, chloroform was found to be the best for complete extraction of the complex. The colour of the extract remained stable for at least 72 h at room temperature (28°C).

Beer's law was obeyed over a range of 0.05 to 3 ppm of iron, with an optimum concentration range, evaluated from Ringbom's plot, of 0.1–2.75 ppm of iron. The Sandell sensitivity of the ion-pair complex was found to be 0.019 μg iron cm^{-2}. At 480 nm the molar absorptivity is $2.9 \times 10^4\, l\, mol^{-1}\, cm^{-1}$. The precision of the method calculated from relative standard deviation of 10 measurements is found to be ± 1.5%.

The probable stoichiometry of the complex was investigated by the Job's method of continuous variation and the equilibrium shift method. Job's method indicated a ratio of 1:1 for iron to CPZ, and the ratio of thiocyanate to iron, from the equilibrium shift method, was 4:1. Hence the ratio Fe:SCN:CPZ in the extracted ion-pair is 1:4:1.

The effect of presence of several extraneous cations and anions on the extraction of the ion-pair of iron was tested by adding different amounts of foreign ions to a fixed amount of iron. The colour was developed and extracted, and absorbance was measured following the general procedure described above. Cations of alkali and alkaline earth metals did not interfere. Among the transition metals, Mo(VI), Nb(V), Re(VII), Co(II) and Ti(IV) interfered seriously. However, interference due to Nb(V) was reduced by masking it with NaF during extraction, while that due to Re(VII) was reduced by its pre-extraction with tetraphenylarsonium ion. Molybdenum must be separated before extraction. Among the anions, F^-, Cl^- and Br^- at up to 5000 ppm had no effect on the absorbance, and phosphate did not cause interference. Thiosulphate and I^- interfered.

To ascertain the validity of the proposed method, standard steel samples H. No. 6-4828 58 Cr V_4 and H. No. 7-2034 15 C DV_6 were analysed. The result showed values of 96.7 and 96.1% against the certified values of 96.78 and 96.292 respectively. Haematite ore analysis by the proposed method indicated a value of 63.1%, as against the certified value of 63.56%.

3 September 1988; Revised 14 December 1988

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A RAPID SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF VANADIUM(V) IN SEDIMENTARY ROCKS

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THE colorimetric methods available for the determination of vanadium in traces are lengthy, tedious and involve the removal of interfering ions by solvent extraction[1-3]. L-α-Methyl-3, 4-dihydroxyphenylalanine[4] or α-methyldopa (known as M-dopa, Alphadopao or Sembrina commercially) is a pharmaceutical preparation (British Pharmacopoeia)[5] used in the treatment of hypertension. In the present studies it was observed that M-dopa forms a purple-coloured complex with vanadium(V) in acidic medium and can be used as a colorimetric reagent.

The chemicals used were of analytical grade. All measurements were done in triplicate. Absorption spectra (visible light) were recorded on a Carl Zeiss VSU-2 spectrophotometer. Ammonium metavanadate (AMV, 2.296 g) was dissolved in distilled water (1 l) to give a concentration of 1000 ppm vanadium. M-dopa (10 mg) was dissolved in 0.1 N H_2SO_4 (50 ml), this gives a concentration of 200 μg/ml. Solutions B of rock samples were prepared by employing the standard method of Riley[6]. Clay fraction was fused with HF and $HClO_4$ in a platinum crucible and the residue was dissolved in distilled water.

Aliquots of standard AMV were taken in measuring flasks and diluted with distilled water to make different concentrations ranging from 10 to 100 ppm vanadium. These solutions were pipetted out (2 ml) into a series of stoppered test tubes. To these test tubes M-dopa solution (2 ml) was added. An unstable pink colour developed immediately. The reaction mixture was then heated on a steam bath for 10 min and cooled to room temperature. The stable purple-coloured complex formed had absorption maximum (λ_{max}) at 560 nm (figure 1). The developed colour was stable for up to 12 h.

Absorbance was measured for different concentrations of vanadium at 560 nm using reagent as blank. Beer–Lambert law was obeyed up to 500 ppm of vanadium (figure 2). Molar absorptivity of the complex was $2 \times 10^2\ \mathrm{l\ mol^{-1}\ cm^{-1}}$ at 560 nm. The method is quite reproducible (SD + 2 ppm, $P < 0.05$). The working pH range was observed to be between 1 and 3.

Most of the common ions present in rocks do not interfere with the determination of vanadium(V) by this method. However, to evaluate the tolerance limit of different ions, synthetic solutions containing 100 ppm V(V) and different amounts of various ions were prepared. Tolerance limits in ppm (error ± 2%)

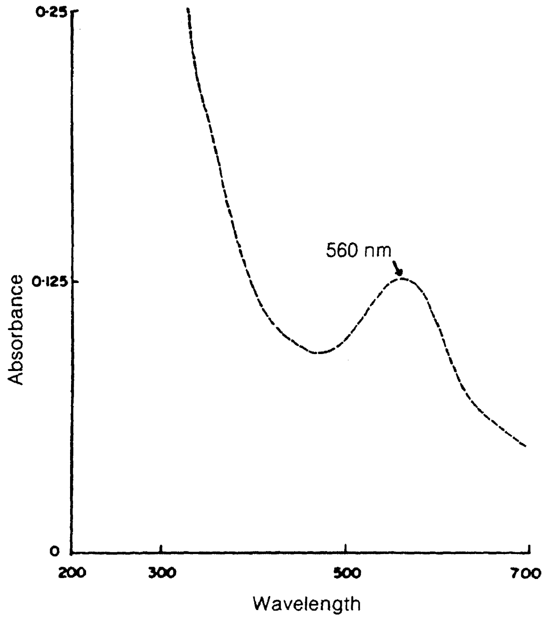

Figure 1. Absorption spectrum of vanadium complex with α-methyldopa (1-cm cell).

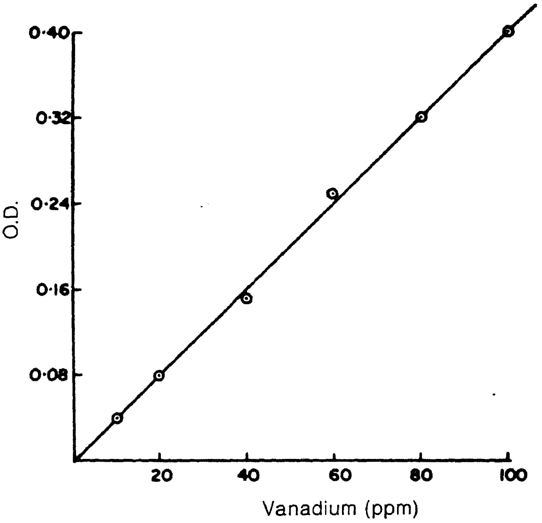

Figure 2. Calibration graph for vanadium using α-methyldopa (1-cm cell, 560 nm).

for different metal ions are given in brackets below: Ca(II) (2000), Mg(II) (1000), Sr(II) (1000), Na(I) (2000), K(I) (1500), Fe(III) (500), Mn(IV) (1000), Ti(IV) (1200), Ga(III) (50), Ni(II) (500), Cu(II) (500), Zn(II) (500), Co(II) (200), B (200) and Cr(III) (150).

The reaction mixture was heated gently on a steam bath. It was observed that rapid heating leads to the formation of a brownish complex or precipitation of the purple-coloured complex. Use of strong concentrated acids may also lead to precipitation of the complex.

To an aliquot of solution B of rock sample, an acidic solution of M-dopa was added and the reaction mixture was heated in the manner described for standard vanadium samples. The results for a few samples analysed by this method were compared with those of Majumdar and Das[2] using *N*-benzoyl-*o*-tolylhydroxylamine, and are summarized in table 1.

It has been reported earlier[7] that vanadium(V) forms a green-coloured complex with absorption maximum at 680 nm with M-dopa. In fact M-dopa and vanadium(V) form a purple-coloured complex with λ_{max} at 560 nm (figure 1). To examine the earlier reported method, various concentrations of M-dopa and vanadium(V) were mixed in separate tubes and heated for different periods in acidic medium. A green-coloured complex with absorption maximum at 680 nm could never be obtained.

The molar absorptivity and Sandell's[8] sensitivity of the reaction are $2 \times 10^2 \, \mathrm{l\,mol^{-1}\,cm^{-1}}$ and 0.035 $\mu g/cm^2$ respectively. The Job's[9] method and mole ratio method suggest that a 1:2 complex is formed between metal and the reagent. The $\log k$ was found to be 8.86 at 25+0.5°C, pH 2.0.

It seems that vanadium(V) in acidic solution forms VO_2^+ ions which in turn react with $-NH_2$ and $-COOH$ groups of the phenylalanine moiety of M-dopa to give a distorted octahedral complex.

Table 1 *Vanadium analysis data for sedimentary rock samples from Bhima Basin*

	Present method		*N*-Benzoyl-*o*-tolyl-hydroxylamine method[2]	
Sample No.	OD at 560 nm	Vanadium (ppm)	OD at 510 nm	Vanadium (ppm)
BV-I	0.27	67	0.84	70
BV-7	0.24	60	0.70	58
BV-II	0.30	75	0.90	75
BV-14	0.32	80	0.94	78
BV-25	0.38	95	01.15	96

Cell- 1 cm.

Attempts to dissociate the vandadium–M-dopa complex are in progress and a detailed study to elucidate its structure will be carried out.

5 May 1988

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A NOTE ON THE RADIOACTIVE INTRAFORMATIONAL CONGLOMERATE IN PAKHAL BEDS NEAR BANGARUCHILKA, KHAMMAM DISTRICT, ANDHRA PRADESH

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BANGARUCHILKA (17°46′15″ N; 80°36′46″ E), is a small village located about 5 km north of Mailaram Copper Mines in Khammam district, Andhra Pradesh (T.S. No. 65 C/9). The area exposes schists and gneisses of the Dharwar age and acid intrusives. Acid intrusives include pink granite and associated pegmatites and vein quartz. All these are unconformably overlain by Pakhal/Gondwana sediments. The radioactive intraformational conglomerate occurs within quartzite of Pakhal Supergroup in Turpu Gutta, about half a kilometre due north-east of Bangaruchilka village.

Field data indicate that the radioactive intraformational conglomerate is a polymictic conglomerate, consisting mainly of pebbles of quartz and lesser feldspars, with a length of up to 18 cm and length/breadth ratio of 1.5–2.0. The conglomerate passes into gritty arkose and finally to arkose. It is

Monazite

exposed intermittently over a total strike length of about 2 km, with width varying from 5 to 40 m. It is a sheared conglomerate, in which pebbles are oriented in NNE–SSW direction without any plunge. Matrix is silicious and sericitic, with pebble to matrix ratio ranging from 2:1 to 1:2.

Under the microscope, the conglomerate shows a lot of sub-rounded quartz with undulatory extinction. Feldspars include predominant microcline and minor plagioclase. The pebbles are fractured. Heavy minerals include zircon, apatite, rutile, goethite. sphene, monazite and thorite. A major portion of the radioactivity is contributed by thorite and monazite. Thorite is euhedral, while monazite is irregularly shaped.

The conglomerate and its variants record radioactivity of the order of 2 to 10 × bg. Grab samples from them have analysed %e U_3O_8, % U_3O_8 and % ThO_2 in the range of 0.023 to 0.069, 0.005 to 0.018, and 0.044 to 0.14 respectively.

10 October 1988; Revised 5 December 1988

MICROPORELLUS MURR. (POLYPORACEAE): A NEW GENERIC RECORD FROM INDIA

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AN interesting polypore, *Microporellus obovatus* (Jungh.) Ryv., was collected from Cherrapunji on one of several trips in the Khasi, Jaintia and Garo hills (Meghalaya). *Microporellus* is morphologically similar to the widely distributed genus *Microporus* but the latter differs anatomically in having a trimitic hyphal system, the presence of typical coralloid elements in hymenium, and in having allantoid to cylindric-ellipsoid basidiospores. The collections have been deposited in the Herbarium, Department of Botany, Panjab University, Chandigarh (PAN).

Microporellus obovatus (Jungh.) Ryv., *Norw. J. Bot.*, 19(3–4): 232, 1972. Figures 1–3.

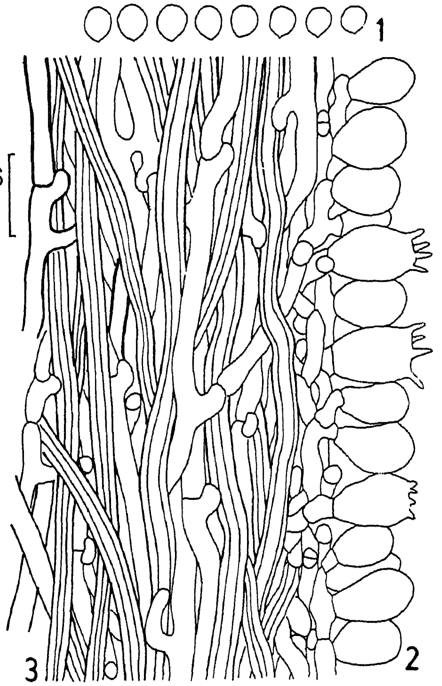

Figure 1. *Microporellus obovatus* (1) Basidiospores; (2) hymenium with clavate basidia; (3) part of **trama** showing dimitic hyphal system.

Fructification annual, laterally stipitate, solitary or in small groups of two to four with fused pilei and free stipes, soft and coriaceous when fresh, hard and brittle on drying. Pileus flabelliform to spathulate, up to 5 cm long, 3 cm wide and 2 mm thick; upper surface light brown to yellowish brown to greyish brown, darkening on drying, finely tomentose when young, glabrous due to agglutination of hyphae with age, radially striate on drying; margin papery, thin, sterile below, brown, incurved on drying. Stipe up to 2.5 cm long and 1–2 mm thick, yellowish brown, finely velvety to glabrous, concolorous with pileus, smooth when fresh, radially wrinkled on drying, expanded gradually into a mycelial disc at the base, solid. Pore surface cream to light brown when fresh, entire; pores small, round to rarely angular, thin-walled, 6–7 per mm, 60–96 µm in diameter; dissepiment entire, 24–78 µm thick; tubes in one layer, brown, 1.4 mm deep in section. Context thin, brown, homogeneous, non-xanthochroic, up to 0.6 mm thick.

Hyphal system dimitic; generative hyphae hyaline to subhyaline, thin- to thick-walled, branched, septate, clamped, 1.5–4.5 µm in diameter; skeletal hyphae hyaline to subhyaline, thick-walled, aseptate, unbranched, 2.8–4 µm in diameter. Cystidia absent.

Wood rot

Basidia clavate, hyaline, 4-spored, 9–11 × 6–7.5 μm. Basidiospores hyaline, thin-walled, smooth, globose to subglobose, non-amyloid, 3.2–4.6 × 3–4.2 μm.

Collection examined: India, Meghalaya, Cherrapunji, Mawsmai falls, on decaying angiospermic stump, S. S. Virdi 21864, 21865 (PAN), August 16, 1984.

This is the first report of the occurrence of *M. obovatus* in India. The species seems to be restricted in its distribution to the Khasi hills since various explorations in the eastern Himalayas and adjoining hills yielded the above cited collections from Cherrapunji. The description of the species as given above matches closely with that given by Ryvarden and Johansen[1]. The species is distinguished by laterally stipitate, coriaceous fructifications; spathulate to flabelliform pilei; fine, tomentose, brown to reddish brown upper surface; dimitic hyphal system; and hyaline, thin-walled, globose to subglobose basidiospores.

The author thanks DST, New Delhi, for financial assistance and Prof. K. S. Thind for encouragement.

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FARINOSE SPOT OF FRENCH BEAN—A NEW DISEASE

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FRENCH bean (*Phaseolus vulgaris*) has gained popularity as a pulse crop in Sikkim in the recent past although it has been grown for a long time as a vegetable. During a disease survey, an apiphytotic of farinose spot was found in November 1987 at elevations between 1000 and 1350 m above MSL. The disease caused heavy defoliation; about 65% of the lower leaves were affected.

The disease symptoms first appear on the lower leaves as yellowish spots and progress upward. In severe infection these appear as yellow mottling. In old infection the spots become brown with yellow margin. These spots are generally 2–3 mm in diameter, but several spots might merge to affect most of the leaf area. On the underside of these spots, white, fluffy growth of the fungus can be seen. The farinose growth was more prominent on the lower surface of the fallen leaves. The infection was confined to the leaves only. Premature defoliation causes heavy yield loss. However, this disease was not observed in summer and rainy-season crop.

The causative organism was identified as *Mycovellosiella phaseoli* (Drummond) Deighton. The pathogenicity was established by spraying spores and mycelial suspension on leaves. The typical disease symptom appeared within 7 to 10 days. The diseased specimen has been deposited in the herbarium of CMI, Kew, England, under reference no. IMI 322046. A perusal of the literature[1] indicated that this fungus has not been reported earlier, and therefore this is a new record from India.

The authors are grateful to the Director, CMI, Kew, England, for identification of the fungus.

30 September 1988; Revised 13 December 1988

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A MOSAIC DISEASE OF SISSOO (*DALBERGIA SISSOO* ROXB.)—A NEW RECORD

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DURING a field survey around Agra in 1986–87, mosaic symptoms were seen on leaves of sissoo. The symptoms were suspected to be viral in origin, and on review of the literature it was observed that there was no record of any virus disease on this plant. Therefore studies were carried out on this virus in an insect-proof glasshouse following methods described earlier[1, 2]. The inoculated sissoo and *Chenopodium amaranticolor* plants were kept in the insect-proof glasshouse at 30 ± 2°C and were observed regularly.

On sissoo, chlorotic, circular spots appeared 9–11 days after inoculation. When inoculation was done at 4–5-leaf stage these spots gradually became irregular and changed into a mosaic pattern.

Infected plants were stunted and their leaves were greatly reduced in size. Distinct, countable, chlorotic, circular lesions were produced on *C. amaranticolor* 9–11 days after inoculation. These turned necrotic with a pale halo after 18–20 days. The disease could also be transmitted to healthy sissoo plants by cleft grafting. Transmission studies have clearly indicated the aetiology of the disease of sissoo to be viral in nature. This virus disease is being reported for the first time from India.

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SCANNING ELECTRON MICROSCOPIC STUDIES ON HILUM STRUCTURES IN BLACK AND GREEN GRAMS

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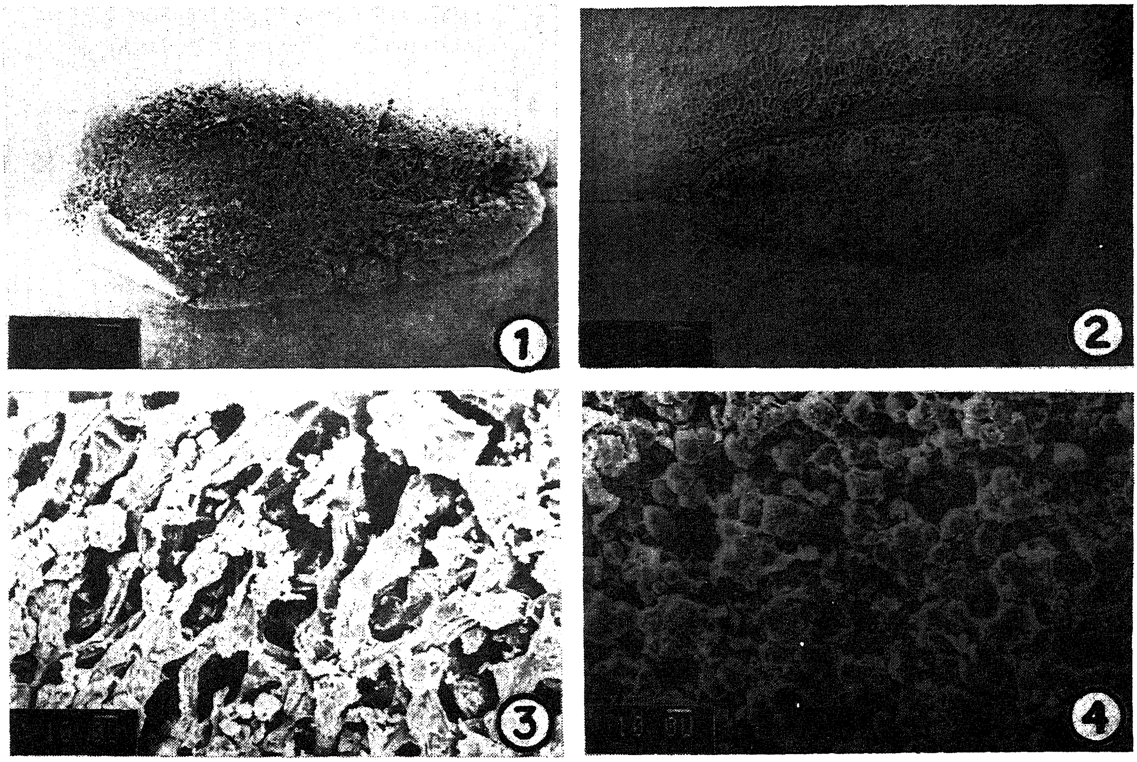
THE genus *Vigna* (Phaseoleae:Papilionoideae:Leguminosae) has many cultivated species, of which *Vigna mungo* (L.) Hepper, former synonym *Phaseolus mungo* L. (black gram), and *Vigna radiata* (L.) Wilczek, former synonym *Phaseolus radiatus* L. (green gram), are most important grain legumes of the Indian subcontinent[1]. They differ from each other in many morphological features, colour of seed and shape of hilum being the main distinguishing features[2-4]. Scanning electron microscopy (SEM) has been employed to study testa topography by many workers[5-9]. However, the hilum has remained uninvestigated. The present SEM studies deal with



Figures 1–4. SEM photographs of hilum of seeds of *Vigna mungo* and *V. radiata*. **1,** *V. mungo*: ovate and concave hilum with thick and raised hilar rim (× 54); **2,** *V. radiata*: lanceolate and flat hilum without any rim (× 54); **3,** *V. mungo*: reticulo-tuberculated pattern (× 540); **4,** *V. radiata*: simple tuberculated pattern (× 540).

the microstructural details of the hilum.

Seeds of *V. mungo* and *V. radiata* were procured from G. B. Pant University of Agriculture and Technology, Pantnagar. Two or three randomly selected seeds were mounted on a brass stub and coated with a thin film (200Å) of gold in a JFC-1100 ion sputter coater. The coated specimens were examined in a JEOL-JSM-35C microscope at an accelerating potential of 15 kV. Photographs were taken using 120 mm film.

In *V. mungo*, the hilum is concave and ovate. Besides, a thick and raised rim is also present around the hilum (figure 1). The hilum of *V. radiata*, on the other hand, is flat and lanceolate without any rim. The hilar region is, however, demarcated from the testa by a narrow groove (figure 2).

With regard to the microstructural details, the hilar region of *V. mungo* revealed reticulo-tuberculated pattern, the tubercles being situated unevenly over the loosely knitted reticulae (figure 3). In case of *V. radiata*, the hilum was simply tuberculated without any reticulation. The tubercles were, however, larger than those of *V. mungo* and found scattered evenly throughout the hilar region (figure 4).

Based on the earlier SEM studies on seed coat structures in the species under investigation, many conclusions have been drawn. Sharma *et al.*[5] pointed out the role of surface characters in solving taxonomic and phylogenetic problems. Kumar and Rangaswamy[10] could report that the seed surface of *Vigna* was species-specific. Trivedi and Gupta[8,9], while studying the seed coat structures, concluded that the shape of the hilum was significant from the taxonomic point of view.

The present SEM studies are of taxonomic and phylogenetic utility. It may be concluded that *V. mungo* and *V. radiata*, having distinct hilum microstructures, are separate species. Besides, the presence of tubercles in the hilar region of both the species could suggest their origin from a common ancestral stalk—the view also shared by Zukovskij[11], Dana[12] and Jain and Mehra[1].

The authors are grateful to Dr P. V. Sane, Director, NBRI, Lucknow, for providing SEM facilities. One of the authors (MIAK) thanks CSIR, New Delhi for a fellowship.

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MECHANISM OF LEAF ROLLING IN RICE

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LEAF rolling is one of the earliest visible physiological responses of plants to water deficit[1]. In rice (*Oryza sativa* L.) it begins at relatively high leaf water potential and progresses across a wide water potential range[2]. Although leaf rolling has often been explained as a means of reducing transpiration rate by plants experiencing water deficit[3], quantitative experimental data were provided only recently[4]. The mechanism of such drought-induced leaf rolling, however, has not been systematically studied. This communication provides information on leaf rolling initiation, progression and completion in response to field moisture stress in 100 genotypes of rice.

These rice genotypes, with a range of maturity duration, were grown under unirrigated rainfed upland conditions at the Crop Research Station (Masodha), Faizabad, during 1982 and 1983. Plants experienced moisture stress during dry spells that occurred at different growth stages. This allowed assessment of leaf rolling at vegetative, jointing, advanced reproductive and anthesis stages. The adaxial and abaxial surfaces of leaves were examined in the field and microscopically in the laboratory. A definite and precise variation in the smoothness of the leaf surface on either side of the midrib of adaxial and abaxial sides of leaves was noticed in all the genotypes. The leaf surface on one side of the midrib was smooth and on the other ridged and rough. These halves are referred to as 'smooth portion' and 'ridged portion' respectively (figure 1A). The ridged portion has many long and prominent strips running parallel to the midrib but the smooth portion has either no such prominent strips or has very few and rudimentary ones. The occurrence of smooth and ridged portions on leaves of a given plant was always phyllotaxically symmetrical. Whenever a leaf experienced water deficit the ridged portion bent first inward, followed by the smooth portion rolling over the former, resulting in a tubular shape of the leaf (figure 1B, transverse view).

Four types of leaf rolling initiation were identified in upland rices during field moisture stress. Rolling that originates at the tip and progresses towards the leaf base is common during vegetative to jointing stage when soil moisture stress develops slowly. Rolling of entire leaf also occurs, with ridged and smooth portions bending almost simultaneously; the time between initiation and completion of rolling is quite short. This type of leaf rolling occurs after jointing or during reproductive growth stages when moisture stress develops rather suddenly due to either water deficit in the soil or increased evaporative demand of the atmosphere or both. Helical rolling, often confined to the region from the tip to the middle of the leaf, and resulting in a twisted tubular shape occurs in the flag leaf during anthesis when stress develops rapidly. Further progression of helical rolling towards the leaf base gives rise to a non-helical shape on completion of rolling. This type of rolling is often observed in the flag leaf prior to anthesis when stress develops slowly.

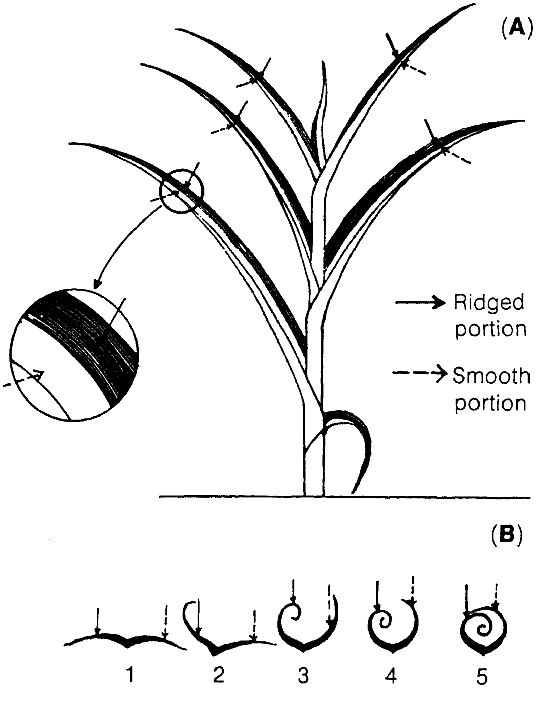

Figure 1. **A,** Ridged and smooth portions on rice leaves. **B,** Sequence of events in leaf rolling.

The reason for the ridged portion rolling first, followed by the smooth one, might be differential loss in their turgor, i.e. turgor reduction occurs first in the ridged portion from the margin side, which is farther from the xylem vessels of the midrib, and slowly extends towards the midrib, and is followed by loss of turgor in the smooth portion, which then bends over the ridged one which has already rolled in. The reason for the lower turgor of the ridged portion is not known. However, it could be that the distribution of specialized bulliform cells (motor cells) is unequal, these cells being more in the ridged portion compared to the smooth, and this results in rapid water loss. Such a possibility may be arrived at from observations on differential rolling in sorghum and maize leaves reported by Martin[5]. Although the reasons for the existence of ridged and smooth leaf surfaces aiding leaf rolling during drought are not known, their identification in itself is an important and interesting observation from the standpoint of leaf rolling in rice.

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RESIDUAL MERCURY ACCUMULATION IN EXPOSED MULBERRY PLANTS

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THE hazards of uncontrolled release of industrial and agricultural wastes have been clearly revealed by several serious incidents of mercury poisoning[1,2]. In each case the deaths were caused by consumption of food contaminated with high levels of mercury and pesticides incorporated through biological magnification in food chains. Considerable attention has been focused on mercury and mercury-based pesticides. Some of the mercury-based pesticides find use in mulberry cultivation. The literature on residual accumulation of mercury and its effects on mulberry plantation is scanty. The present work was undertaken to study the residual accumulation of mercury by the use of mercury-based pesticides and mercury-containing solid waste on mulberry plants.

A branch of mulberry (*Morus alba*) was cut into pieces 10 inches long. These were planted in different pots containing 6 kg manured soil (manure to soil 1:2). Care was taken to avoid flooding or drying of the pots. Pesticides and solid wastes were applied after the sprouting of 8–10 leaves on each mulberry cutting.

Solid waste of a chlor-alkali industry containing mercury was applied in one set of experiments. The solid waste contained 748.6 ± 32.3 mg of mercury/kg dry weight. Six concentrations were prepared, viz. 1.5, 3, 4.5, 6, 7.5 and 9%, and were applied in 6 pots at a time.

In the second set, $HgCl_2$ was applied. The application of the chemical was by two routes, i.e. application through the soil and by foliar spray. In one group of pots, $HgCl_2$ was applied through the soil and in another, $HgCl_2$ was applied by foliar spray. Three different concentrations of $HgCl_2$, viz. 0.02, 0.50 and 2 mg/l, were employed in each group.

In the third set, Emisan-6 at 1000, 2500 and 5000 mg/l was applied through the soil and by foliar spray. The concentrations selected were based on the studies of Mohapatra[3]. A set of control plants were maintained for comparison in each case.

Leaves from both control and treated plants were collected. The treated leaves were washed thoroughly to remove materials adhering to the waxy coat. A set of leaves was processed separately for dry weight determination.

Residual mercury was measured with a (cold vapour atomic absorption) mercury analyser using $HgCl_2$ (Analar) as the standard following the basic principle of Wanntrop and Dyfverman[4], which has undergone substantial modification in the light of recent developments[5]. The process of analysis has been described elsewhere[6].

Figure 1 shows residual concentrations of accumulated mercury ($\mu g/g$ dry wt) in leaves after application of different concentrations of chlor-alkali solid waste, $HgCl_2$ and Emisan-6.

At lower doses of the solid waste there was a low amount of retention. With increase in solid waste concentration the residual mercury level showed a significant increase and at 9% the highest value (2.06 ± 0.32 $\mu g/g$ dry weight) was recorded 30 days after exposure.

There were significant differences in residual concentrations between application of $HgCl_2$ by foliar spray and application through soil. The recorded residual level of Hg on leaves after foliar spray of 0.2 mg/l $HgCl_2$ was 120.16 ± 8.16 $\mu g/g$ dry weight, and at 2 mg/l of $HgCl_2$, the residual Hg was 139.42 ± 18.42 $\mu g/g$ dry weight. Soil application gave corresponding residual mercury levels on leaves of 3.7 ± 0.5 $\mu g/g$ and 3.90 ± 0.33 $\mu g/g$ dry weight.

In the case of Emisan-6 the differences in residual Hg levels between foliar spray and soil application were similar to those in the case of $HgCl_2$.

Control plants did not show any trace of mercury in the leaves.

It has been reported earlier[7–9] that mercury can be accumulated in different biotic systems to a dangerous level. Uptake and accumulation of chemicals may prove to be most important aspect of pollution dynamics. Since the leaves of the mulberry plant are the food of silkworm larvae, accumulation of mercury and pesticides in the leaves can cause damage to the sericulture industry. Larvae fed on contaminated leaves accumulate the pollutants within the body. Mercurial compounds are known to affect the growth and efficiency of animals[7,9]. It can be presumed that because of the drastic decline in growth and metabolism silk production by the

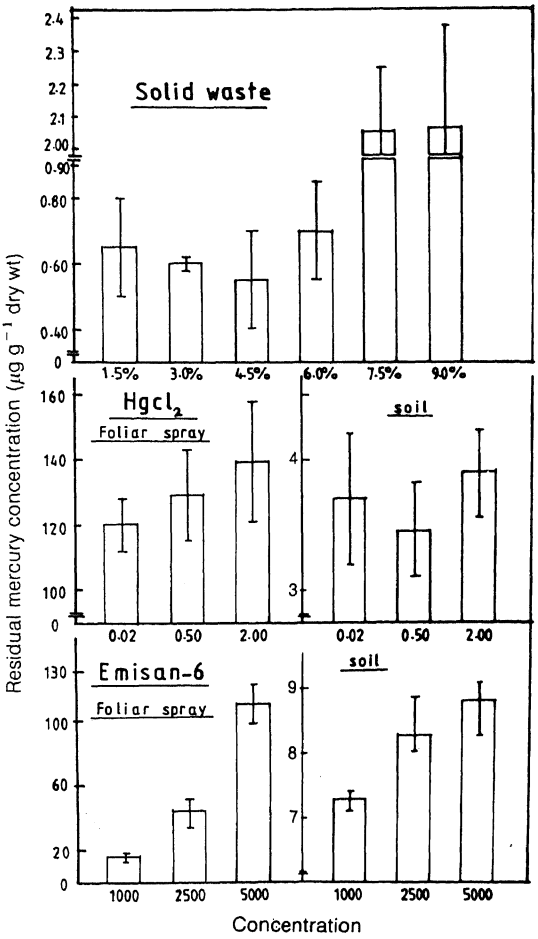

Figure 1. Residual mercury concentration (µg/g dry wt) in leaves of treated mulberry plants 30 days after application. Bars show mean of 10 samples; standard deviations are also shown.

larvae will be affected and the silk fibre may be very weak and of low quality.

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INHIBITION OF PHOTOSYNTHESIS IN LEAF DISCS BY HERBICIDES

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HERBICIDES are known to inhibit photosynthesis at metabolic sites[1]. Recently, work on photo-inhibition by herbicides at low concentrations on leaf discs has been described[2].

Developed leaves of rice (cv. IET-7633), maize (cv. VL-16), soybean (cv. Lee) and groundnut (cv. JL-24) were collected from field-grown plants grown with recommended levels of nutrients. Leaf discs (1 cm diameter) were treated overnight in different concentrations of simazine (0, 3, 6, 9 and 12 ppm), butachlor (0, 80, 160, 400 and 800 ppm) and glyphosate (0, 80, 160, 400 and 800 ppm). Photosynthesis inhibition was estimated on the basis of permanent submergence of leaf discs placed afloat in 0.05 M $NaHCO_3$ solution in light[2].

Photosynthesis inhibition by the herbicide simazine was more pronounced in soybean and groundnut leaf discs than in rice and maize (table 1). Fifty per cent inhibition was recorded in rice and maize at a concentration of 12 ppm. But at that concentration, the inhibition in soybean and groundnut was 70%. This is in agreement with the results of earlier workers[3,4]. Simazine effects are by inhibition of electron transport, and disorganization and rupture of the tonoplast and chloroplast envelope[5,6].

Table 1 *Per cent inhibition of photosynthesis by simazine, butachlor and glyphosate in rice, maize, soybean and groundnut leaf discs*

Herbicide	Concentration (ppm)	Rice (IET-7633)	Maize (VL-16)	Soybean (Lee)	Groundnut (JL-24)
Simazine	0	—	—	—	—
	3	—	25	33	—
	6	—	37	50	30
	9	20	45	60	60
	12	50	50	70	70
Butachlor	0	—	—	—	—
	80	20	35	20	20
	160	40	45	50	50
	400	70	50	70	60
	800	90	60	100	70
Glyphosate	0	—	—	—	—
	80	—	35	60	—
	160	30	45	70	—
	400	40	50	80	20
	800	60	60	100	60

Butachlor showed a decreasing order of inhibition in soybean, rice, groundnut and maize at higher concentrations. However, at 80 ppm the adverse effect was maximum in maize leaf disks. Similarly the total weed killer glyphosate showed the highest degree of inhibition in soybean (80%). Inhibition in these cases is mainly at the PS II system through uncoupling or inhibition of electron acceptance and transport mechanisms as well as inhibition of CO_2 uptake[4].

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GENETIC VARIATION AT ALCOHOL DEHYDROGENASE LOCUS IN SOME DROSOPHILIDS

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MEASURING the patterns and amounts of genic variation in natural populations of diverse organisms is the major thrust of experimental population genetics[1-3]. Allozymic (allelic isozyme) variations detected by gel electrophoresis have been used to assess the extent of genetic variability in species populations. Alcohol dehydrogenase (ADH, EC 1.1.1.1) constitutes an important gene–enzyme system in *Drosophila* because of its role in detoxification and/or utilization of alcohol in the natural habitat of the organism[4]. Several field and empirical studies have been made on ADH polymorphism in *D. melanogaster* but information about this enzyme in other drosophilids is scanty[5-7]. The present investigation was undertaken to examine the extent of electrophoretic variation of ADH in some drosophilids.

Individuals of species *D. melanogaster*, *D. takahashii*, *D. nepalensis*, *D. malerkotliana*, *D. bipectinata*, *D. ananassae*, *D. jambulina*, *D. punjabiensis*, *D. immigrans*, *D. busckii* and *Zaprionus indianus* were bait-trapped from Delhi, Rohtak, Pinjore, Jammu. Hasimara, Bagdogra and Dhulabari (Nepal). Labo-

Gel electrophoresis

ratory cultures of *Z. sepsoides* and *Z. tuberculatus* were also employed. Wild caught males and F_1 individuals from species-specific isofemale lines were analysed electrophoretically. In order to compare electrophoretic patterns, individuals belonging to different subgenera (*Sophophora*, *Dorsilopha* and *Drosophila*) were included in the same gel. Each horizontal 12% starch gel slab measured 15 × 10 × 1 cm and could accommodate 12–14 samples. Homogenates of single individuals were subjected to electrophoresis at 250 V and 25 mA at 4°C for 3.5 h. Gel slices were stained for the related and overlapping enzyme systems ADH, octanol dehydrogenase (ODH) and aldehyde oxidase (AO) by standard staining procedures[8,9]. The gel slices stained for ADH revealed three zones of activity due to non-specificity and overlapping band patterns of alcohol-oxidizing enzymes (ADH, AO and ODH). On the basis of comparison of gel slices stained for ADH, AO and ODH, it was found that ODH and AO constitute the two anodal zones while the single cathodal zone is true ADH. Genetic control of ADH electromorphs or banding patterns in each species was interpreted from the segregation patterns of ADH electromorphs of parents, and F_1 and F_2 progeny of several single-pair matings. The genetic interpretation of banding patterns and calculation of genetic indices such as allelic frequency, heterozygosity and effective number of alleles were done following a standard source[10]. The log-likelihood χ^2 test (*G*-test) was used to assess whether the observed genotypes were in agreement with those expected on the basis of Hardy–Weinberg equilibrium.

The electrophoretic mobility patterns of ADH are species-specific (figure 1). The ADH zymograms of three species (*D. jambulina*, *D. punjabiensis* and

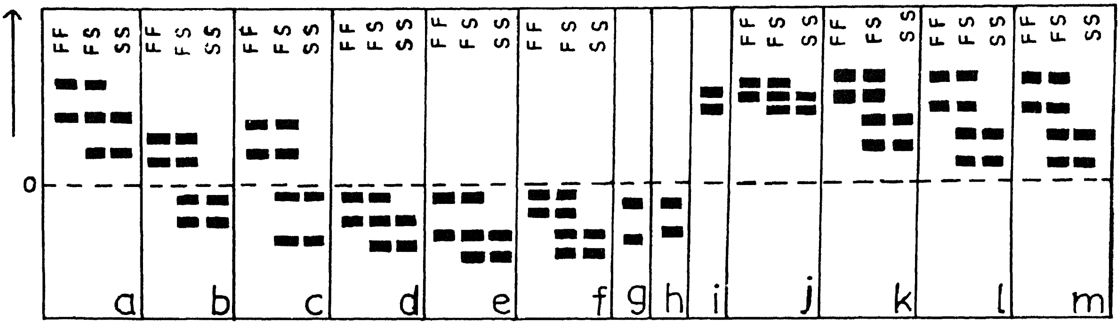

Figure 1. Electrophoretic phenotypes of ADH in homogenates of single individuals of drosophilids (a–m). In species polymorphic for ADH, band patterns in first and third slots represent different homozygous genotypes (FF and SS) while that of the second slot represents heterozygous ADH genotype (FS). Differential binding of coenzyme (NAD) causes two-band patterns in homozygotes. a, *D. melanogaster*; b, *D. takahashii*; c, *D. nepalensis*; d, *D. malerkotliana*; e, *D. bipectinata*; f, *D. ananassae*; g, *D. jambulina*; h, *D. punjabiensis*; i, *D. immigrans*; j, *D. busckii*; k, *Z. indianus*; l, *Z. sepsoides*; m, *Z. tuberculatus*.

Table 1 *Inheritance patterns of polymorphic alcohol dehydrogenase electromorphs in ten drosophilids. Genetic crosses were intra-species*

ADH phenotype of genetic cross	No. of matings	ADH phenotype of progeny			Sample size	Test for Mendelian ratios	χ^2*
		FF	FS	SS			
FF × SS	2	—	46	—	46	—	—
FS × FS	3	28	66	34	128	1:2:1	0.75
FS × SS	2	—	38	44	82	1:1	0.43
SS × FF	1	—	27	—	27	—	—
FS × FF	2	33	29	—	62	1:1	0.26
FS × FS	2	35	79	38	152	1:2:1	0.47
SS × FS	1	—	18	22	40	1:1	0.40

FF and SS are fast and slow electrophoretic variants represented by two-banded patterns; *Non-significant at 5% level.

Table 2 *Observed ADH genotypes, allelic frequencies, heterozygosities, effective numbers of alleles (n_e) and G values (log-likelihood χ^2 test) for Hardy–Weinberg expectations at the ADH locus in different drosophilids*

Species	ADH genotypes							Sample size	Allelic frequencies				Heterozygosity		n_e	*G* value
	1,1	2,2	3,3	4,4	1,2	2,3	3,4		1	2	3	4	Obs.	Exp.		
D. melanogaster	8	60	—	—	40	—	—	108	0.26	0.74	—	—	0.37	0.38	1.62	0.14
D. takahashii	—	84	6	—	—	24	—	114	—	0.84	0.16	—	0.28	0.27	1.36	2.85
D. nepalensis	—	116	12	—	—	48	—	176	—	0.80	0.20	—	0.27	0.32	1.47	4.3
D. malerkotliana	—	—	8	70	—	—	36	114	—	—	0.23	0.77	0.31	0.36	1.55	1.10
D. bipectinata	—	—	12	54	—	—	40	106	—	—	0.30	0.70	0.38	0.42	1.73	1.19
D. ananassae	—	—	16	60	—	—	48	124	—	—	0.32	0.68	0.38	0.44	1.77	1.61
D. jambulina	—	—	109	—	—	—	—	109	—	—	1.0	—	—	—	1.0	—
D. punjabiensis	—	—	102	—	—	—	—	102	—	—	1.0	—	—	—	1.0	—
D. immigrans	121	—	—	—	—	—	—	121	1.0	—	—	—	—	—	1.0	—
D. busckii	4	92	—	—	4	—	—	100	0.06	0.94	—	—	0.04	0.11	1.13	18.4*
Z. indianus	6	113	—	—	86	—	—	205	0.24	0.76	—	—	0.41	0.38	1.57	3.91
Z. tuberculatus	16	32	—	—	40	—	—	88	0.41	0.59	—	—	0.45	0.48	1.94	0.31
Z. sepsoides	16	26	—	—	30	—	—	72	0.43	0.57	—	—	0.41	0.49	1.96	1.71

*Significant at 1% level; Other *G* values are non-significant.

D. immigrans) show two-banded phenotypes. Electrophoretic analysis of parents and progeny of several single-pair matings in these species revealed that the two-banded ADH phenotypes do not show any segregation behaviour and thus represent a monomorphic zone of ADH activity. In the other ten species, the species-specific homozygous phenotypes are represented by two-banded ADH patterns of either faster mobility (FF) or slower mobility (SS) (figure 1). Segregating two-banded (FF and SS, with one band of each having the same mobility) and three-banded patterns were observed in four species (*D. melanogaster*, *D. malerkotliana*, *D. bipectinata* and *D. busckii*). Genetic crosses involving segregating two-banded patterns in these species resulted in three-banded patterns in F_1 and a 1:2:1 ratio of segregating two-banded and three-banded patterns in F_2 progeny. The other six species showed two-banded (FF and SS, with both bands of each having non-overlapping mobilities) and four-banded patterns (figure 1). In these species, genetic crosses involving segregating two-banded patterns resulted in four-banded patterns in F_1 and a 1:2:1 ratio of segregating two-banded and four-banded patterns in F_2 progeny (table 1). The electrophoretic data for these crosses are in agreement with monogenic control of ADH pattern. Thus homozygous individuals showing different two-banded patterns/electromorphs are allozymic variants. The observed ADH isozyme patterns are in sharp contrast to many other gene–enzyme systems where a single band represents an allelic isozyme or allozyme[2,3]. The present observations on ADH patterns in different drosophilids agree with other reports on *D. melanogaster*, i.e. in homozygous strains more than one electromorphs/bands may arise because of differential binding of the coenzyme NAD[11-15].

The distribution of ADH genotypes, allelic frequencies, observed and expected heterozygosity, effective number of alleles and log-likelihood χ^2 test for fit to Hardy–Weinberg expectations at the ADH locus in various drosophilids are given in table 2. The ADH locus is effectively polymorphic in ten drosophilids on the basis of the criterion that the most common allele does not exceed 0.95. Except *D. busckii*, all the species polymorphic at the ADH locus show a fit to Hardy–Weinberg equilibrium and do not reveal rare alleles. Most of the species show occurrence of two common ADH alleles and high heterozygosity value. The maintenance of two common alleles at the ADH locus in several drosophilids may be explained on the basis of balancing natural selection. However, both field and laboratory studies on several eco-geographical populations of these drosophilids need to be analysed to assess the role played by evolutionary forces in the maintenance of genic diversity at the ADH locus.

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PLANTLET REGENERATION FROM HYPOCOTYL TISSUE OF *STRYCHNOS NUXVOMICA* LINN.

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PLANT tissue culture has wide application in horticulture, agriculture and forestry. Tissue culture is useful in preserving desirable plant characters and in plant improvement[1,2]. It has many advantages over sexual propagation in large-scale reforestation programmes[3,4]. However, success with trees of medicinal importance has still been limited.

Seeds of *Strychnos nuxvomica* Linn. (Loganiaceae) were germinated on Murashige and Skoog (MS) medium[5] supplemented with 1 mg/l gibberellic acid in an Erlenmayer flask under aseptic conditions at 25 ± 1°C in the dark. Segments (3 mm) excised from hypocotyl of the seedlings were inoculated on MS medium supplemented with various growth regulators at different concentrations. Cultures were maintained at 25 ± 1°C with 16-h illumination under fluorescent light (2000–3000 lux).

Hypocotyl segments started proliferating into brown to white calli just after a week of inoculation. Callus initiation started on medium supplemented with 0.1 to 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and was enhanced two-fold after addition of 0.5 to 2.0 mg/l benzyladenine. Callus growth was better with 0.5 mg/l indole-3-acetic acid (IAA) and 0.8 mg/l kinetin in combination. Calli (three to four weeks of age) were subcultured on MS medium without any growth regulator and on media supplemented with different growth regulators. Calli ceased to grow further on medium devoid of any growth regulator but continued to grow in the presence of 0.2 to 2.0 mg/l kinetin. After 10 days the callus turned brownish-green, bearing a few nodules. Shoot primordia developed from the nodular portions of the callus and subsequently produced shoot buds (table 1). These shoot buds further differentiated into shoots within six weeks. Rhizogenesis took place after 15 days of culture in the medium supplemented with 0.1 to 2.0 mg/l naphthaleneacetic acid (NAA), as shown in table 1. However, increase in NAA concentration above 2 mg/l was inhibitory to root formation.

Combination of NAA (0.1 to 1.0 mg/l) and kinetin (0.2 to 2.0 mg/l) also induced shoot bud formation. Media containing 2 mg/l kinetin and 2 mg/l NAA in combination and kinetin alone beyond 2 mg/l failed to produce any organogenesis. NAA (0.1 to 1.0 mg/l) and kinetin (0.2 to 2.0 mg/l) in combination facilitated root formation whereas shoot bud formation was a bit delayed (observed only after 4 weeks), resulting in direct plantlet formation in certain cases (table 1). Plantlets grew well, attaining a height of 5 cm in eight weeks in medium containing kinetin (2 mg/l) and NAA (1 mg/l) in combination.

In vitro regeneration of *S. nuxvomica* Linn. was

Table 1 *Morphogenesis in response to various growth regulators in the hypocotyl callus of Strychnos nuxvomica Linn.*

Added growth regulator (mg/l)			Morphogenesis (%) ± SD			Number of plantlets per culture
Kinetin	NAA	IAA	Shoot buds	Roots	Plantlets	
0	0	0	0	0	0	0
0.2	0	0	22.0 ± 1.6	0	0	0
1.0	0	0	28.0 ± 1.0	0	0	0
2.0	0	0	26.0 ± 1.0	0	0	0
3.0	0	0	0	0	0	0
0	0.1	0	0	20.0 ± 1.0	0	0
0	0.5	0	0	25.0 ± 2.0	0	0
0	2.0	0	0	10.0 ± 1.0	0	0
0.2	0.1	0	15.0 ± 2.5	0	5.0 ± 1.6	1
2.0	0.5	0	20.0 ± 2.0	0	15.0 ± 1.0	3
2.0	1.0	0	0	0	20.0 ± 2.0	3
0.2	0	0.5	0	0	0	
0.5	0	1.0	0	0	0	

Each treatment consisted of 50 cultures. Data were recorded at the end of 50 days. The experiment was repeated thrice.

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*For correspondence.

obtained because of balance among growth regulators. It is well established that auxin–cytokinin balance facilitates organogenesis and is necessary for the regeneration of plants in a culture[6–9]. In this plant, of the various cytokinins used kinetin produced the maximum shoot buds, whereas among auxins NAA was the best for root formation.

19 March 1988

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ASSOCIATION OF GEMINIVIRUS-LIKE PARTICLES WITH YELLOW MOSAIC DISEASE OF *DOLICHOS LABLAB* L.

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YELLOW mosaic symptoms were observed on the leaves of *Dolichos lablab* L. in Lucknow. Similar symptoms have been reported from Pune[1], and whitefly (*Bemisia tabaci* Genn.) has been established as a vector of the disease agent[2]. We report here the transmission of the disease agent and type of particles associated with the disease. For whitefly transmission, the method detailed earlier[3] was used for acquisition access feeding (AAF) and inoculation access feeding (IAF). The whiteflies were allowed AAF of 24 h followed by an IAF of 96 h. Tests were carried out by using *D. lablab* both as donor and recipient host. Twenty to twenty-five whiteflies were used per test plant. Insects were killed by spraying an insecticide after IAF and plants were transferred to an insect-proof glasshouse for 5 to 7 weeks. Back indexing was done 6–8 weeks after inoculation in the manner described earlier. *D. lablab* plants showing typical yellow mosaic symptoms were harvested 4 weeks after inoculation with whiteflies and homogenized with 0.1 M phosphate buffer, pH 8.0, containing 1% 2-mercaptoethanol and 0.01 M sodium ethylenediaminetetraacetate (1 : 3). The extract was squeezed through cheesecloth, clarified at 8000 rpm for 20 min, and 4% polyethylene glycol (PEG) and 1.5% sodium chloride were added. The mixture was incubated at 4°C for 3 h and centrifuged at 10,000 rpm for 20 min. The pellets were resuspended in 0.01 M phosphate buffer, pH 7.5. The suspension was clarified at 8000 rpm for 10 min, stirred with 2.5% Triton X-100 for 1 h at 4°C, and centrifuged for 10 min at 10,000 rpm. The supernatant thus obtained was loaded on 10 ml of 20% sucrose, prepared in phosphate buffer, and centrifuged at 35,000 rpm for 2 h in a Beckman Ti 45 rotor. Pellets were resuspended in 5 ml PB, centrifuged at low speed (10,000 rpm) for 10 min and finally at high speed (40,000 rpm) for 2 h in a Beckman SW 50.1 rotor. The pellet was resuspended in 2 ml of buffer and subjected to a low-speed centrifugation for 10 min at 8,000 rpm. Linear sucrose gradients (10–40%) were prepared in 0.1 M phosphate buffer, pH 7.0, containing 0.001 M EDTA. The partially purified preparation (0.5 ml) was layered on the gradients and centrifuged at 40,000 rpm for 2.5 h in a Beckman SW 50.1 rotor. Two distinct light-scattering bands observed were collected and concentrated by pelleting at 40,000 rpm for 2 h. The samples thus obtained were stained with 2% uranyl acetate (pH 4.0) and examined under a Philips 410 transmission electron microscope. Particles were measured with an X7 Bausch and Lamb magnifier directly from negatives.

During purification of the particles two light-scattering bands were observed after 10–40% sucrose density gradient centrifugation. The UV absorption spectrum of the lower band was typical of nucleoproteins with maximum absorbance at

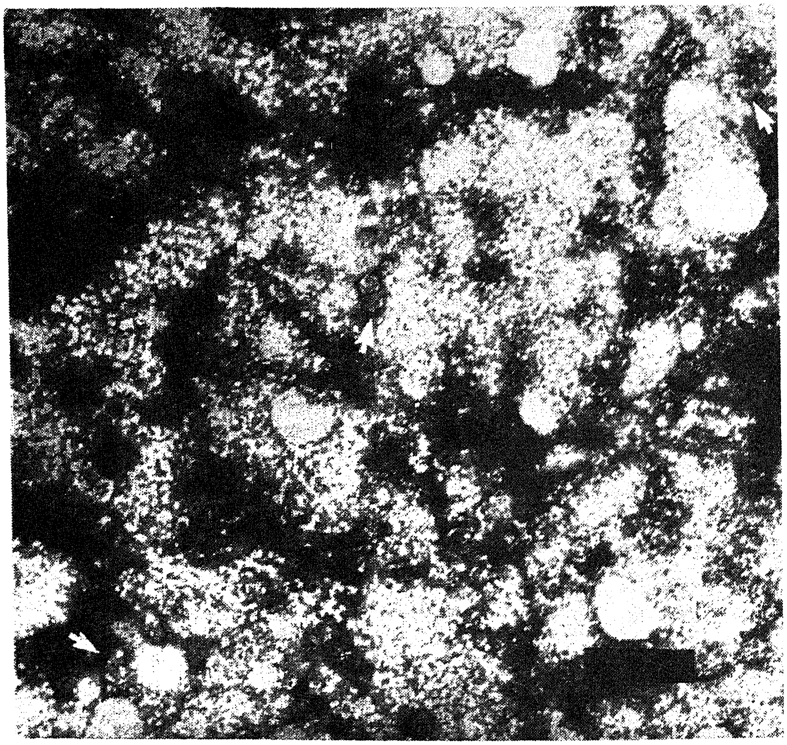

Figure 1. Electron micrograph of geminivirus-like particles stained with 2% uranyl acetate. Arrows indicate dimers. Bar represents 100 nm.

258 nm and minimum at 242 nm. The A_{260}/A_{280} ratio was 1.46 (uncorrected for light scattering), similar to that of several geminiviruses[4]. Electron microscopy of the sample obtained in the upper band revealed the presence of 12-nm particles, whereas 20 × 40 nm germinate particles were found in the lower band (figure 1). However, the intact geminate particles were only a few in number (1 or 2 particles per field) but most of the particles appeared to be degraded. The smaller number of geminate particles may be due to their susceptibility to the 2% uranyl acetate used for staining, as geminiviruses have been reported to be very susceptible to negative stains[5]. Particles of 12 nm appear to be phytoferritins.

Whiteflies transmitted the disease but at a very low transmission rate (2/50). These findings confirm the earlier report[2], where only 3% transmission of the disease was achieved by *B. tabaci.*

The yellow mosaic disease agent exhibits the characteristics shared by some suspected or proven geminivirus group members, including whitefly transmissibility and particle morphology[6,7]. The geminiviruses may be responsible for the yellow mosaic in *D. lablab*. However, for unequivocal proof of their involvement in the disease, an extensive investigation is needed.

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24 May 1988; Revised 14 September 1988

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MICROBIAL CONVERSION OF MURRAYANINE TO MUKOEIC ACID

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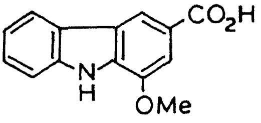
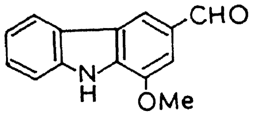
MUKOEIC acid[1], a carbazole alkaloid isolated from *Murraya koenigii*, has been assigned structure (I) based on physical evidences and also based on its formation from murrayanine (II)[2,3]. The biological oxidation of murrayanine to mukoeic acid was suggested based on the occurrence of both of them in the same plant[4]. The present study was undertaken to investigate microbial means of conversion of (II) into (I).

Initially, we searched for an appropriate organism capable of oxidizing an aromatic aldehyde to its corresponding acid, since the natural product II contains an aromatic aldehyde group. As a model system, we investigated the possibility of microbial oxidation of salicylaldehyde (III), a commercially available compound. For this purpose, strains of *Pseudomonas aeruginosa*, *Escherichia coli* B and *Bacillus firmis* were grown in medium (pH 6.8) containing KH_2PO_4 1 g, K_2HPO_4 2 g, $CaCl_2$ 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KNO_3 1 g, NaCl 0.2 g, yeast extract 0.3 g, and glucose 10 g per litre. Salicylaldehyde was added to a concentration of 250 μg/ml after 24 h of growth. After incubation for another 48 h the culture filtrates were extracted with ethyl acetate. On removal of solvent, only the *Pseudomonas* culture filtrate yielded a white solid. The solid was crystallized from aqueous alcohol. The crystalline compound (yield 60%), m.p. 159°C, was identified as salicylic acid (IV) from its physical and chemical properties and by direct comparison with a pure sample of salicylic acid.

In a similar study *P. aeruginosa* was also found to be quite efficient in converting compound II to I. Maximum conversion could be achieved 24 h after addition of the substrate to a final concentration of 150 μg/ml to the culture. The culture filtrate was extracted with ethyl acetate. On removal of the solvent a semi-solid mass was obtained. It was then chromatographed over silica gel using a series of solvents of increasing polarity, and three compounds were isolated. One was identified as the unconverted compound (II). The second compound (yield 50%), m.p. 241°C, has been assigned molecular formula $C_{14}H_{11}NO_3$ (M^+ 241). The UV spectrum (λ_{max} 236, 270 and 320 nm log ε 4.50, 4.58, 3.92) and IR spectrum (ν_{max} 3431, 1690, 1635, 1615, 1610 cm^{-1}) of the compound were strikingly similar to those of mukoeic acid (I), and identity was finally confirmed by direct comparison with natural mukoeic acid (m.m.p., UV, IR). The other compound, m.p. 228°C, molecular formula $C_{13}H_9NO_3$ (M^+ 227), gave violet coloration with $FeCl_3$, indicating the presence of phenolic hydroxyl group. The UV spectrum (λ_{max} 240, 266, 286, 315 and 323 nm, log ε 4.59, 4.64, 3.8, 3.78, and 3.18) bears a strong similarity to that of 1-hydroxycarbazole with carboxyl substitution at the 3 position[5]. The IR spectrum indicated it was an aromatic substance with an NH (3430 cm^{-1}), one hydroxyl (3200 cm^{-1}) and one carboxylic acid group (1690 cm^{-1}). From all these data the compound has been assigned the structure V.



(I)



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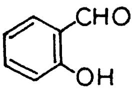
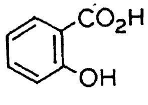


(III)



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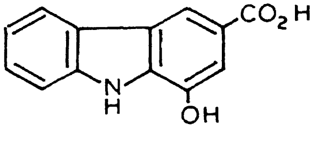

(V)

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ACETYLENE REDUCTION ACTIVITY OF SOME BLUE-GREEN ALGAE

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MOST aerobic nitrogen-fixing blue-green algae are filamentous forms and N_2 fixation takes place in differentiated cells called heterocysts which serve as N_2-fixing factories[1]. Certain N_2-fixing, heterocystous blue-green algae develop associations with algae, fungi, bryophytes, the water fern *Azolla*, gymnosperms and the angiosperm *Gunnera*[2]. These associations are of interest because the algae supply fixed nitrogen to the host plant and are, in many ways, analogous to rhizobia[3]. Relative to free-living isolates, symbiotically associated blue-green algae have a five-fold or even higher frequency of heterocysts[4, 5]. The present paper describes the acetylene reduction activity (ARA) of six blue-green algae in relation to their heterocyst frequency and age. These estimates would serve as a reference for inoculum selection, growth and biochemical studies.

The blue-green algal isolates used are listed in table 1. Axenic cultures of the blue-green algae were grown in BG-11 medium[6] under continuous light at 50 μmol photons $m^{-2} \cdot s^{-1}$ at 25°C. The cultures were harvested after 3, 7 and 30 days, washed with sterile BG-11 medium[7] and suspended in the same medium. ARA was measured by the acetylene reduction assay[7]. The cultures (5 ml each) were placed in 15 ml serum vials with 10% (v/v) acetylene as the gas phase at 25°C and a photon flux of 250 μmol $m^{-2} \cdot s^{-1}$. Gas samples (1 ml) in two replicates were collected after 30 min and analysed in a gas chromatograph (model Tracor 540). The heterocyst frequency was calculated as per cent of total cells by light microscope observations of the filaments. Chlorophyll *a* was estimated by the method of Mackinney[8].

Table 1 shows the data on relative ARA and heterocyst frequency of six blue-green algal strains. Based on their ARA values and heterocyst frequency, the algae can be separated into two main groups: one with high ARA and high heterocyst frequency (*Nostoc anthoceros, Anabaena cycadeae*) and the other with low ARA and low heterocyst frequency (*Anabaena* 7120, *A. variabilis, A. doliolum* and *Nostoc linckia*). The free-living isolates from *Anthoceros* and *Cycas* had high ARA values (4.47 and 4.04 nmol C_2H_4 per μg Chl *a* per hour respectively) compared with the other isolates. This can be explained by the fact that these isolates had average

Table 1 *Acetylene reduction activity and heterocyst frequency of blue-green algae*

Isolate	Source	Acetylene reduction activity* (nmol C_2H_4 per μg Chl *a* per hour) Young culture (3-day)	log-phase culture (7-day)	old culture (30-day)	Heterocyst frequency (%)
Anabaena doliolum	Isolated from paddy field (BHU)	1.47	3.38	0.586	4.95
Anabaena 7120	Gift from B. Bergman (Uppsala, Sweden)	1.42	2.98	0.284	4.55
Anabaena variabilis	Gift from A. K. Kashyap (BHU)	1.29	3.39	0.691	4.65
Nostoc linckia	—”—	1.51	3.60	0.713	4.72
Anabaena cycadeae	Original isolate from corralloid root of *Cycas circinalis* (BHU)	1.71	4.04	1.29	6.00
Nostoc sp.	Original isolate from *Anthoceros* gametophyte (Shillong)	1.88	4.47	1.59	8.90

*Average of six independent experiments.

Nitrogen-fixation

heterocyst frequency of 8.9 and 6% respectively, compared with values of 4–5% in the other isolates. This observation further substantiates the importance of heterocysts in nitrogen fixation. Fairly young (3-day) and very old (30-day) cultures showed lower ARA than those in log phase (7-day), suggesting that the enzyme nitrogenase is synthesized significantly more in the log phase. ARA was also altered by changes in medium and culture conditions (data not shown), suggesting that the history of cultivation and age of cultures play an important role in the regulation of ARA (nitrogen fixation) in blue-green algae.

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TROPANE ALKALOIDS FROM *LYCIUM BARBARUM* LINN., *IN VIVO* AND *IN VITRO*

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TROPANE alkaloids, which are pharmacologically important compounds, have been reported from solanaceous plants such as *Atropa*[1,2], *Hyoscyamus*[3,4], *Datura*[5,6], *Duboisia*[7] and *Scopolia*[8]. However, there is no report on the production of tropane alkaloids from intact plant parts and in tissue culture of *Lycium barbarum* of an Indian arid zone. Therefore the present investigation was undertaken.

Plant materials (roots, shoots and fruits) were freshly collected from Davi Kund Sagar, Bikaner. The voucher specimen of *L. barbarum* was collected from the Department of Botany, University of Jodhpur, Jodhpur and deposited at the same place. Unorganized tissue of *L. barbarum* was established from seedlings on Murashige and Skoog's[9] medium supplemented with 5 ppm of kinetin. The growth index (GI) of each of the tissue samples was calculated from

$$\mathrm{GI} = \frac{\text{Final dry weight of tissue} - \text{initial dry weight of tissue}}{\text{Initial dry weight of tissue}}$$

The various plant parts and 6-week-old tissues at maximum GI (7.5) were dried and ground to a fine powder. The powdered material was extracted with chloroform for 24 h. Thin-layer chromatography (silica gel G; chloroform: methanol: ammonia, 30:60:2) gave two spots in all the plant parts and tissue samples tested. The spots were visualised under UV light (254 nm) and developed by spraying with modified Dragendorff's reagent[10] and heating at 100°C until the characteristic colours developed. The spots corresponded with reference atropine (R_f 0.35, dark orange) and hyoscyamine (R_f 0.36, light orange). The alkaloids present in the extracts were obtained by preparative (0.4 to 0.5 mm) TLC on silica gel plates eluted with chloroform. Each isolated compound was crystallized and further analysed for m.p., m.m.p. and IR spectra, and compared with the authentic sample for confirmation. The various extracts were subjected to quantitative estimation by the procedure of Feldman and Robb[11] for atropine and the method of Gaur[4] for hyoscyamine. Five replicates were examined in each case, and mean and 95% confidence limits obtained.

The callus tissue was hairy, compact in texture, and whitish-green in pigmentation. The maximum GI observed was 7.5 in a six-week-old tissue. Tropane alkaloids in each of the plant parts and in 6-week-old tissue at maximum GI were identified as atropine and hyoscyamine. Atropine and hyoscyamine were confirmed by m.p. (atropine, 117–118°C and hyoscyamine, 108°C), m.m.p. (undepressed) and IR spectroscopy.

Total alkaloid was nearly the same in shoots and fruits lower in calli, and still lower in roots (table 1). Fruits had the highest atropine content and shoots the highest hyoscyamine content.

Table 1 *Tropane alkaloid content (per cent dry weight) of Lycium barbarum in vivo and in vitro*

Alkaloid	Alkaloid content* (%) Roots	Shoots	Fruits	Callus
Atropine	0.42 ± 0.07	0.93 ± 0.02	0.95 ± 0.04	0.74 ± 0.02
Hyoscyamine	0.25 ± 0.02	0.33 ± 0.02	0.29 ± 0.01	0.09 ± 0.05
Total alkaloid	0.67 ± 0.04	1.26 ± 0.02	1.24 ± 0.02	0.83 ± 0.03

*Mean and 95% confidence limits.

Mitra[1] and Khanna *et al.*[2] have estimated the atropine content of roots (0.45%) and seedling callus cultures (0.53%) of *A. belladonna* respectively. Hocking[3] showed the presence of alkaloids in roots (0.08–0.11%), stems (0.01–0.025%), leaves (0.04–0.08%), flower tips (0.07–0.10%), seeds (0.06–0.10%) and whole herbs (0.02–0.08%) of *H. niger*. Gaur[4] showed significant amounts of hyoscine (0.019%) and hyoscyamine (0.57%) in seedling callus cultures of *H. niger*. Tropane alkaloids have also been reported from the seeds (0.32%) of *D. innoxia*[5]. Prabhakar *et al.*[6] have described the commercial production of tropane alkaloids such as hyoscine from the leaves and seeds of *D. innoxia*, *D. metal* and *D. fastuosa*.

The present results show that yields of tropane alkaloids from tissue culture are lower than those from intact plant parts and are dependent on added growth factors in the medium.

The fruits of *L. barbarum* are widely eaten by camels and goats in the Indian arid zone. Studies on the effects of these alkaloids on these animals will be interesting.

L. barbarum growing in Indian arid zone is a new source of tropane alkaloid. The presence of high amounts of atropine in the fruits of the intact plant is of great commercial importance and merits the attention of the pharmacological industry.

The author is grateful to Prof. (Miss) P. Khanna, Department of Botany, University of Rajasthan, Jaipur, and to Dr T. N. Nag, Lecturer in Botany, Dungar College, Bikaner, for providing laboratory facilities and valuable guidance. The investigation was supported by financial assistance from CSIR, New Delhi.

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PRELIMINARY REPORT ON CARDIAC DEPRESSANT EFFECT OF *HEMIDISCUS HARDMANNIANUS* (BACILLARIOPHYCEAE)

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In recent years, investigations of marine plants and animals for useful drugs have become increasingly important. At present no diatom has been shown to be a potential source of drugs other than antibiotics. *Hemidiscus hardmannianus* is a common diatom in the Indian seas. It was tested for possible cardiac activity.

H. hardmannianus was collected from the mouth of

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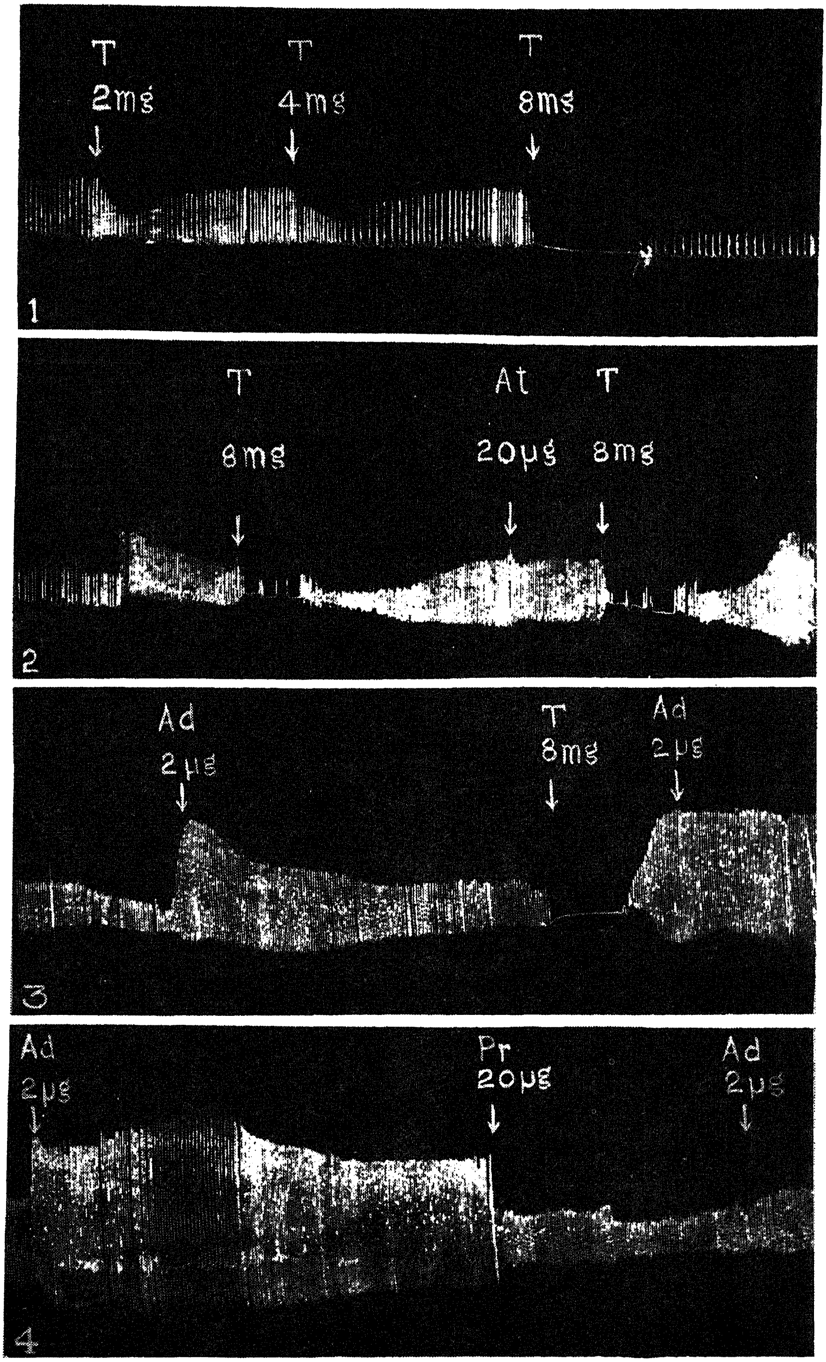
*For correspondence.

Cardiac activity



Figures 1–4. **1,** Dose-related cardiac depressant effect. **2,** Cardiac depressant effect of the test extract before and after atropinization. **3,** Blockade of adrenaline stimulation by the test extract. **4,** Propranolol blockade upon adrenaline stimulation. [Ad, Adrenaline; At, atropine; Pr, propranolol; T, test extract.]

the Vellar estuary (11°29′ N; 79°46′ E). The F/2 medium of Guillard[1] was used for culture of the diatom. The culture was maintained in 25‰ salinity at 29 ± 1°C under illumination of 4000 lux in a 12 h light and 12 h dark cycle. The cultured cells were harvested after they reached stationary phase (7th day). Cells (4.46×10^6) were extracted with hot methanol and the extractions were fractioned with diethyl ether, 1-butanol and water[2]. Only the water-soluble fraction, which was shown to be toxic[3], was used in the experiment. The water-soluble fraction was dialysed and the dialysate was evaporated to dryness in a vacuum evaporator.

Aqueous solution (10 mg/ml) of the substance was prepared for the isolated-heart experiment[4]. Frog's heart was isolated and perfused with Clark's frog Ringer solution[5] at pH 7.4. The effect of different concentrations of the test extract on the heart was recorded. The depressant effect of the test extract was compared with that of acetylcholine using atropine as the blocker. Similarly the depressant effect of the test extract was compared with that of propranolol, a beta-blocker, using adrenaline as the agonist. The experiment was repeated ten times. All the results were consistent.

The cardiac depressant effect of the test extract is dose-related (figure 1). The test extract produced cardiac depressant effect even after atropinization (figure 2), indicating a non-cholinergic nature of action. Figure 3 shows the effect of the test extract during the response to 2 μg of adrenaline. It is clear that the test extract produces initial inhibition followed by its own partial agonistic effect, and then blocks the effect of adrenaline-induced stimulation, unlike propranolol which has no partial agonistic action (figure 4). The test extract may be a beta-blocker with associated partial agonistic activity. Further investigation is in progress.

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MODULATION OF PROTECTIVE EFFECTS OF VITAMIN C IN AFLATOXICOSES

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WIDESPREAD infestation of food and feed by toxigenic strains of *Aspergillus flavus*[1,2] is known to cause mild to severe aflatoxicoses. The damage can be histopathologic[3,4], carcinogenic[5] or mutagenic[6]. In order to minimize the hazards of aflatoxins, a search was made for various drugs that could nullify toxin-caused damage. The well-known antitoxicant, L-ascorbic acid (vitamin C)[7,8], was tested and the results are presented here.

The rate of increase in body weight of weaning guinea pigs (*Cavea cavea*), was used as a parameter for adjudging the general physical profile of the animal. Six- to seven-week-old healthy animals (supplied by Central Drug Research Institute, Lucknow) were fed 50 ± 10 ppb of crude aflatoxin per day per animal along with their normal food for 20 weeks. The method of laboratory elaboration of toxin, maintenance of the experimental animals, and the mode of feeding have been described elsewhere[3,4]. The animals were divided into six groups: (i) no administration of vitamin C or toxin (control), (ii) administration of vitamin C only (AA), (iii) administration of toxin only (AFT), (iv) concurrent administration of toxin and vitamin C (AFT + AA), (v) toxin feeding for 10 weeks followed by exclusive feeding with vitamin C for 10 weeks (AFT→AA), and (vi) vitamin C feeding for 10 weeks followed by exclusive feeding with the toxin for 10 weeks (AA→AFT). The dose of vitamin C was 2 mg/kg body weight which is proportionate with the dose prescribed for humans.

To study the effect on body weight, a weekly mean weight was calculated for each group of animals ($n = 12$ in each group) for up to 20 weeks. From these weekly means, the relative increase in body weight (RIW) was calculated as

$$\text{RIW} = \frac{W_i - W_0}{W_0} \times 100,$$

where W_i and W_0 are mean weight in the *i*th and zero week respectively.

In each group the trend of increase in body weight obtained as RIW was found to be rectilinearly dependent on duration of feeding. Regression equations were calculated and the lines of regression were drawn (figure 1). The results obtained show that the feeding with aflatoxin alone markedly retards the pace of RIW ($y = -1.95 + 4.31\,x$). This retardation is satisfactorily annulled in the group that received aflatoxin and vitamin C concurrently. Some improvement was also found in groups that received vitamin C before or after the toxin.

Though the exact mechanism of action of vitamin C in these situations is not yet known, it can, however, be inferred that a regular oral dose of vitamin C can, to a great extent, minimize the effects of aflatoxicoses.

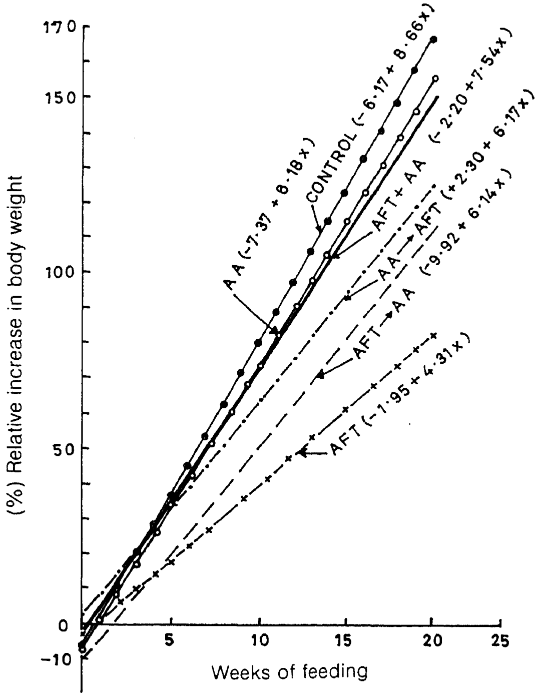

Figure 1. Lines of regression showing weekly relative increase in body weight after toxin and vitamin C treatments. ●——●, Control; ○——○, ascorbic acid only (AA); ———, aflatoxin + ascorbic acid (AFT + AA); ·–·–·–·, ascorbic acid followed by aflatoxin (AA→AFT); – – –, aflatoxin followed by ascorbic acid (AFT→AA); – + – + –, aflatoxin only (AFT).

13 September 1988

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PARASITES OF UZI FLY, *EXORISTA SORBILLANS* WIEDEMANN (DIPTERA: TACHINIDAE): A NEW RECORD

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In recent years five hymenopteran parasites, viz. *Brachymeria lugubris* (Walker), *Dirhinus himalayanus* (Chalcididae), *Nesolynx thymus* (Girault) (Eulophidae), *Spilomicrus karnatakensis* Sharma (Diapriidae), and *Exoristobia philippinensis* Ashmead (Encyrtidae), have been recorded as pupal parasites of uzi fly[1–4], a serious endoparasite of silkworm larvae *Bombyx mori* L.[5] These parasites were earlier reported as primary parasites of Calliphoridae, Muscidae, Sarcophagidae and Tachinidae, and secondary parasites of many lepidopterans through dipterans[6–10].

Recently two more hymenopteran parasites, viz. *Spalangia cameroni* Perkins and *Pachycrepoideus vindimae* (Rondani) (Pteromalidae), were tried on uzi fly maggots and pupae with a view to evaluate their usefulness in biological control of uzi fly[11]. These hymenopterans are usually predominant as house fly parasites[12]. They did not parasitize uzi fly maggots but developed successfully on the pupae. These parasites are easily maintained in the laboratory. Average temperature and humidity recorded in the laboratory were 27±1°C and 72±5% RH respectively. For this study one pair each of male and female parasite were placed with 10 host pupae in each of five glass vials. Parasites were supplied with 50% honey solution as food.

Preliminary observations indicate that *S. cameroni* is a solitary endopupal parasite. Development from egg to adult took 27–29 days. The adult emerged by cutting a circular hole at the anterior end of the host puparium. Sex ratio, male:female, was 1:2.5. A single female parasitizes 1–2 host pupae. Males lived for 6–7 days while females survived for 9–11 days when provided with 50% honey solution.

P. vindimae is also an endopupal parasite. The life cycle was completed in 24–26 days. From a single pupa 3–4 parasites emerged by cutting 2–3 small holes in the wall of the host puparium. Sex ratio, male:female, was 1:4. A single female parasitizes 2–3 host pupae. Males survived for 8–9 days while females lived for 15–17 days when provided with 50% honey solution.

Studies to evaluate the usefulness of these basically house fly parasite types in biological control of uzi fly and to compare their potential with that of the originally reared parasites of uzi fly are in progress.

The authors thank Mr T. M. Manjunath, Biocontrol Research Laboratories, Bangalore, for providing the specimens for study.

29 August 1988; Revised 24 November 1988

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EFFECT OF THE ESSENTIAL OIL FROM THE GUM OLEORESIN OF *BOSWELLIA SERRATA* ROXB. ON THE GONADS OF MALE *DYSDERCUS SIMILIS* F.

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THE role of juvenile hormone (JH) or its analogues in the male reproductive system in insects is not fully understood. However, the effects of JH on testis development and spermatogenesis have been reported in various insects[1,2]. Earlier we have reported JH-mimicking effects (morphological and gonadotrophic)[3] of the gum oleoresin of *Boswellia serrata* on *Dysdercus similis*. In the present communication, the histological derangements in the gonads of male *D. similis* caused by the essential oil of *B. serrata* are reported.

Twenty freshly moulted fifth instar nymphs were taken from stock culture and maintained under controlled conditions at 27±1°C and 70±5% RH. Nymphs were topically applied with *B. serrata* essential oil (1 µl per insect, 1:30 in acetone). The oil has one acyclic monoterpene (myrcene), seven monocyclic monoterpenes (D-α-phellandrene, β-phellandrene, limonene, dipentene, α-terpinene, p-cymene and terpinene-4-ol), and five bicyclic monoterpenes (α-pinene, β-pinene, α-tujene, camphene and bornyl acetate). Controls were treated with pure acetone only. Control and treated insects were fed on water-soaked cotton seeds.

Control and treated insects were dissected in insect Ringer's solution. The reproductive organs

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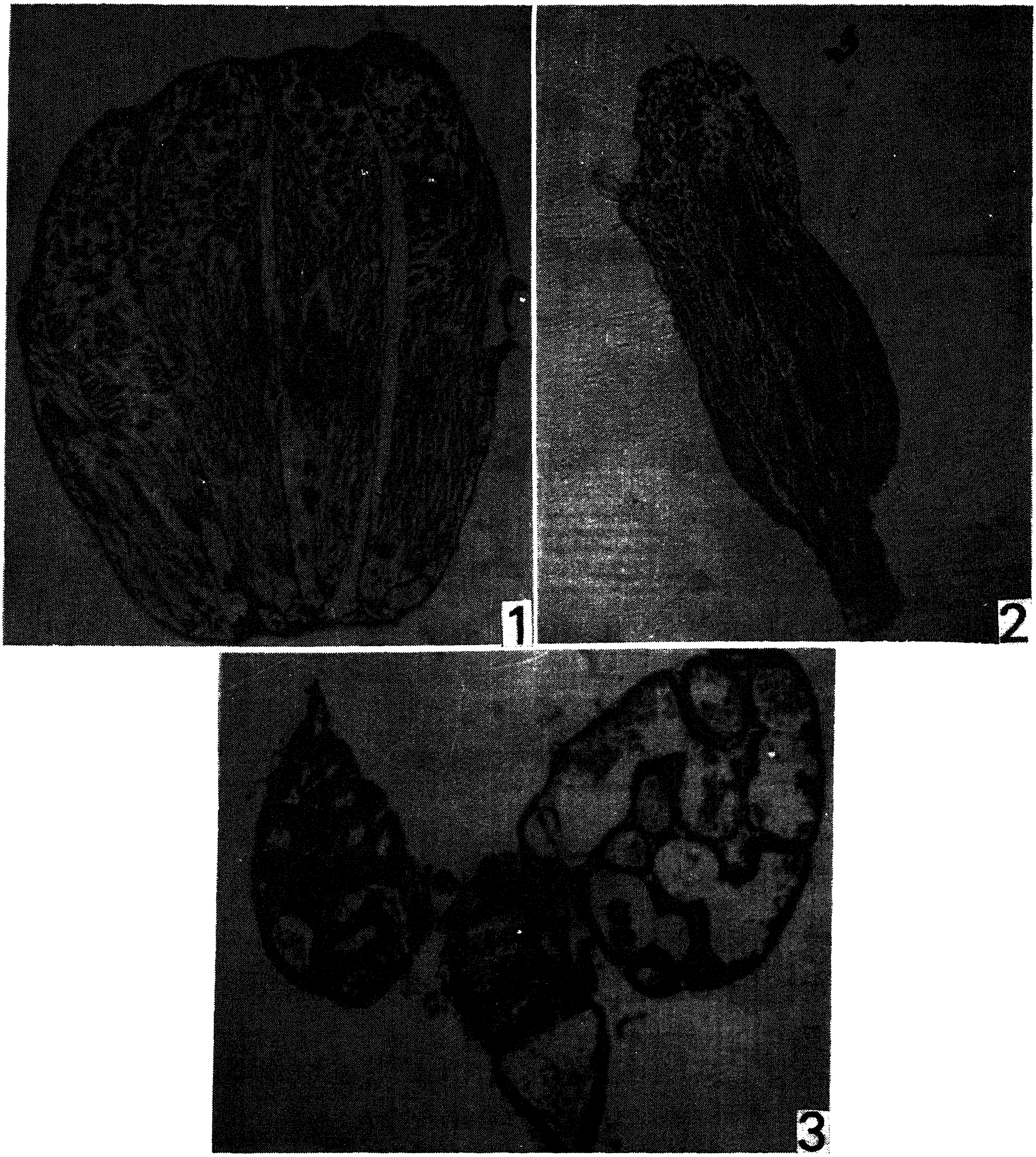
*For correspondence.



Figures 1–3. 1, Sagittal section of testis of treated insect showing degenerated spermatogonia. Note the deformed spermatozoa and their irregular and solitary movement (× 200). **2,** Sagittal section of testis of treated insect showing advanced stage of degeneration. No definite follicles are seen. Note the degenerated spermatogonia and abnormal spermatozoa (× 200). **3,** Section of affected accessory glands showing globules with sparsely distributed secretory material (× 150).

were removed, fixed in Bouin's fluid, dehydrated in an alcohol series, cleared in xylene, and embedded in paraffin wax. Sections (6 μm) were processed and stained in Ehrlich's haematoxylin and alcoholic eosin.

Topical application of the essential oil of the gum oleoresin from *B. serrata* on freshly moulted fifth instar nymphs resulted in production of super-nymphs and adult-nymphs. In the resultant forms, both spermatogenesis and spermiogenesis were seriously affected. This was evident from the various abnormalities observed in the testis, vas deferens and accessory glands.

Sagittal sections of testis showed degeneration of testicular tissue. The spermatogonia lost their cysts and the nuclei became pycnotic and large gaps appeared between them (figure 1). The spermatogonial cells and remnants of the necrotic tissue were found in the apical region of the testis. The heads of the spermatozoa were not prominent and the sperm did not form bundles. In the controls the spermatozoa moved in bundles. The entire testis of treated insects appeared less compact. Distal elongation of testicular follicle and proximal bulging of testes lobes (figure 2) were due to the proliferation of spermatogonia and excessive release of sperm respectively. The swelling of the proximal part of the testis, which was often observed, could be due to the inability of the spermatozoa to enter the thin vas deferens. These effects may be due to the effects of the essential oil on neurosecretory processes. Similar abnormalities were reported by Revathy *et al*[4]. The accessory glands were globular, each lobe surrounded by a thin layer of cells having pycnotic nuclei. The lumen was sparsely filled with secretory material (figure 3). The gland showed inhibition of secretory activity, as shown by the decrease in the amount of secretion[5].

The results show that the essential oil from the gum oleoresin of *B. serrata* Roxb. affects spermiogenesis in *D. similis* F., thereby acting as an effective insect growth regulator.

We thank Prof. R. Nishida, Kyoto University, Japan for a GC–MS examination of the essential oil of *B. serrata*. We thank Prof. M. A. Khan for providing laboratory facilities and Prof. G. Srimannarayana for the sample of essential oil. The authors (DRR and AK) thank ICAR and UGC, New Delhi, respectively for financial assistance.

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SIB-MATING AND REPRODUCTIVE STRATEGY OF *GRYON* SP. (HYMENOPTERA: SCELIONIDAE)

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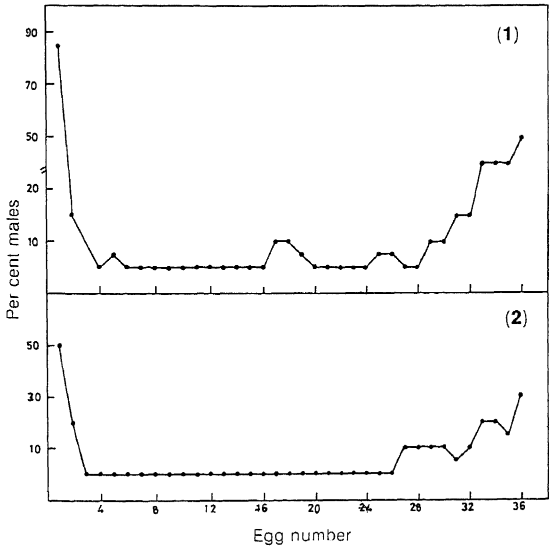
THE search for an effective biocontrol agent against heteropteran insects has often focused on predators and parasitoids and much importance is laid on the egg parasitoids of the family Scelionidae[1-3]. *Gryon* sp. was recorded in India as an effective egg parasitoid of the turpod bug *Clavigralla gibbosa* Spinola[4] and also on the eggs of *Nezara viridula* (Linn)[5]. Apart from *Nezara*, the present study has also recorded this parasitoid from the eggs of *Acrosternum graminea* (Fabricius), a weed-infesting pentatomid. Parasitization by this parasitoid on the eggs of *N. viridula* was 23.24% on an average, while and 35% on *A. graminea*. A comparison of the sex ratios of *Gryon* sp. on these two host eggs, which vary in their number and size, confirms the hypothesis that the sex ratio will decrease with increasing levels of sib-mating[6]. Scelionids appear capable of recognizing eggs parasitized by conspecifics and alter their sex ratio in response to the presence of other wasps.

The present investigation highlights the strategy adopted by the parasitoid in producing a precise sex ratio. A good understanding of these aspects would aid biological control programmes using these scelionids, since the main objective is to maximize the production of mated females and release through laboratory rearing often leads to an increase in the production of males. Eggs of the two hosts were provided in varying numbers and the sequence in which the female parasitoid laid male and female eggs was observed. In all the egg masses, a haploid egg, leading to a male is laid first. By this strategy, every mass, independent of its size, is ensured of a male. In larger masses of eggs (> 25 eggs) a second male is sometimes produced towards the end of the sequence, probably to balance the ratio between males and females, which might be kept track of by the ovipositing parasitoid.

When eggs of varying sizes were offered to the females, the strategy of sequential egg laying was slightly altered. In the case of the smaller eggs of *A. graminea* (diameter 0.75 mm), in 85% of the cases the first egg laid was a male and in about 20% of the cases the second and third eggs were also male. Ten per cent of the fourth and fifth eggs were also males. Thereafter, till the sixteenth egg, 95% of the eggs were female, and then till the 30th egg, 90% were female though a certain amount of variation was observed. From the 31st egg onwards, a higher percentage of male eggs was noticed. The average number of eggs laid by an adult female parasitoid after mating was 33 ± 2 (range 26 to 40) (figure 1). In the case of the larger eggs of *N. viridula* (diameter 1 mm), the observations were similar except that the frequency of female eggs was higher from the third egg onwards. The percentage of males increased from the 26th egg onwards (figure 2). The percentages of male and female eggs were almost equal when an assortment of eggs of the two hosts was offered to the ovipositing female, confirming the fact that under stress a female parasitoid would lay more haploid eggs rather than produce more reproductives (figure 3).



Figures 1 and 2. Allocation of male progeny (eggs) in egg sequence of *Gryon* sp. in host eggs of (1) *A. graminea*, and (2) *N. viridula*. Each point represents frequency of male parasitoid eggs in five runs.

It has been observed that courtship strategies are affected by life-history constraints. In the case of *Gryon* sp., the biological backdrop against which courtship is acted out is that of an 'island' breeding structure, with occasional random mating within almost isolated subpopulations[7]. In scelionids generally, only one female usually parasitizes a host egg mass, but in *Gryon* sp. several females may simultaneously parasitize a group of eggs. Two additional facts are of significance: first, the sex ratio is usually female-biased, and secondly, neither sex tends to disperse until after mating. The females fly away in search of suitable host eggs, whereas the males, which emerge earlier, remain at the place of emergence to mate with subsequently emerging females. Thus these are usually brother–sister matings and the resultant matings for the whole population are actually a mixture of many sib-matings and some occasional outcrosses. Hence, as far as the ability of a male is concerned, the reproductive success of the species depends on the male's capacity to inseminate a large number of females. On the other hand the reproductive success of the species is directly related to the female's capability to locate a suitable host habitat and then the host eggs. It was also observed that within this

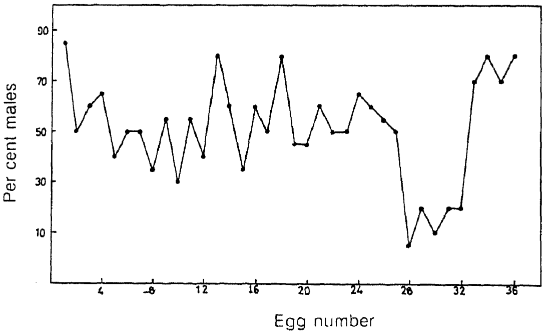


Figure 3. Allocation of male progeny (eggs) in egg sequence of *Gryon* sp. in assorted egg masses of *A. graminea* and *N. viridula*. Each point represents frequency of male parasitoid eggs in five runs.

species the females are more dispersal-oriented and associated changes in behaviour occur soon after mating. This constitutes an important part of the behavioural repertoire by which monandry is maintained. This in turn would be beneficial to the species since it would restrict insemination by the polygynous males to virgin females, thereby maximizing successful reproduction by both sexes within the population. The results indicate the unique reproductive behaviour of *Gryon* sp. that ensures the production of maximum reproductives in the context of the limited reproductive potential of the species. *Gryon* is thus an efficient egg parasitoid of several pests.

The authors are grateful to Prof. T. N. Ananthakrishnan for guidance and encouragement.

27 August 1988; Revised 16 December 1988

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ERRATUM

S. Ramaseshan writes ...

In the printed version of the talk I delivered in Calcutta entitled 'The portrait of a scientist—C. V. Raman' (*Current Science*, **57**, 1207, 1988) there is an error. The sentence reads:

> When the mathematicians of the world presented a copy of Ramanujan's bust to the Indian Academy of Sciences at Bangalore, the astrophysicist S. Chandrasekhar wrote....

The sentence should be replaced by:

> The mathematicians of the world desired to present Mrs Ramanujan with a bronze bust of Ramanujan. The US mathematician Richard Askey persuaded the sculptor Paul Granlund to undertake the making of this bust. Prof. S. Chandrasekhar and his wife Lalitha graciously offered to present one of the copies to the Indian Academy of Sciences. At that time the Chicago astrophysicist wrote....

As reported in the April 1985 issue of *Patrika* (the newsletter of the Indian Academy of Sciences), the unveiling and formal presentation of the bust was done by Mrs Lalitha Chandrasekhar on 6 February 1985 during the Golden Jubilee celebration of the Indian Academy of Sciences. Mrs Lalitha Chandrasekhar then related the story of how the bust came into being—how the sculptor transformed a photograph of Ramanujan (discovered by her husband in India in 1936) into a three-dimensional work of art.

BOOK REVIEWS

Annual Review of Biochemistry, Vol. 57, 1988, pp. 1122, (eds.) C. C. Richardson, P. D. Boyer, I. B. David and A. Meister, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, CA 94306, USA), Price: USA, $35; elsewhere, $39.

The 1988 *Annual Review of Biochemistry* has a prefatory article by F. Sanger entitled 'Sequences, sequences and sequences'. The story of the development of methods for sequencing proteins and nucleic acids has been told in a deceptively simple way. Some of the experiences described by Sanger, who made signal contributions to the progress of biochemistry and molecular biology and won two Nobel Prizes, are worth recording. He states that he is 'ashamed' of only one paper, which was published jointly with A. Tiselius in Uppsala, Sweden, since he (Tiselius) 'had not really contributed anything'—and this in contrast to Chibnall in England, who allowed him to publish a paper on his own even though the work was initiated by Chibnall and the latter could have justifiably put his name. Sanger also discounts the popular idea that 'scientific progress depends on sudden breakthroughs or moments of sudden exhilaration'. Unusually he also 'decided to retire and give up research' on reaching the age of 65 as he thought it would be something of an anticlimax after his successful completion of the DNA sequencing method and having had wonderful opportunities for research for 40 years.

The 28 reviews are a mixture of classical biochemistry and newer aspects of molecular and cellular biology, and have references numbering 100–500. Together they form a resource of work done mostly in the last decade in the areas chosen by the editors for this year. *Annual Review of Biochemistry* continues to be the single most important book to be possessed by all laboratories interested in biochemical research.

DNA can be modified by activated oxygen species, common metabolites of glucose, and diverse inorganic and organic electrophiles, including metals, alkylating agents and polycyclic aromatic hydrocarbons. Yet it is quite stable. Amazingly, a 3.4-kb DNA fragment was cloned from a 2400-year-old mummy! The genetic integrity and functional stability of DNA depends on the repair enzymes. Work on DNA repair has attracted a great deal of attention and has been reviewed under the subjects direct base excision, nucleotide excision, recombination, cross-links, and SOS and adaptive response to oxidative stress. The adaptive response to alkylating agents receives special attention in another review in view of the carcinogenic potential implicit in this process. The mechanism of 'repair of the highly mutagenic DNA lesion O^6-methylguanine' has largely been solved and an 'outline of the regulation of the inducible pathway' is now available. More will be heard in future on the *ada* gene, the regulatory gene of the adaptive response to alkylation damage. This produces a 39-kb Ada protein capable of self-methylation, transferring methyl groups from O-methylated DNA, and as a consequence converting it from 'a weak to a strong activator of transcription'.

The structure and function of bacterial sigma factors, which bind reversibly to the active site of RNA polymerase and play the determining role in the specificity of transcription initiation, are described. The sigma factors form a structurally complex, homologous protein family, sharing the property of binding to the core of RNA polymerase. In another article the current knowledge on transcription by RNA polymerase III, responsible for synthesis of a variety of small RNAs, has been presented.

Eukaryotic chromosomes 'participate in transcription, replication, meiotic and mitotic condensation, pairing, recombination and segregation' through specific 'interactions between nuclear proteins and DNA sequences'. Nuclease-hypersensitive sites in chromatin are the 'open windows' that allow access to *cis*-acting DNA sequences to *trans*-acting factors, and 'occur around centromeres, silencers, recombination sequences, replication origins, upstream activation sequences and promoter elements'.

Another interesting and provocative article covers post-transcriptional regulation of gene expression, especially at the level of translation initiation, elongation and mRNA splicing. The author sees translation initiation as a simple process and the ribosome as 'just a large ribozyme/enzyme' with active sites for its 'substrates', mRNA and fMet-tRNA, with the information necessary for its binding to mRNA contained in a sequence of 35 bases.

The cell needs a balanced supply of the four dNTPs to replicate and this depends on the enzyme ribonucleotide reductase. The enzyme has the general structure $\alpha_2\beta_2$. The α and β subunits have M_r 85,000 and 45,000 respectively, and the small subunit has the characteristic iron-tyrosyl radical centre. The interactions between synthesis of dNTPs and DNA are discussed in considerable detail.

The identification and isolation of *E. coli* DNA polymerase III, the complex enzyme responsible for the replication of the chromosome, was made possible by the use of natural replicative templates in the assay. The current status and future directions of this topic are given in another article.

Proteins and their reactions occupy a central place and this subject is covered in 11 articles. Acylation of specific cellular proteins is ubiquitous. It can occur post-translationally, with fatty acid (primarily palmitate) linked in a thioester or ester bond to cysteine, serine or threonine, or cotranslationally by myristoylation of N-terminal glycine, or carboxy-terminal addition of phosphatidylinositol containing a glycan moiety. The enzyme that catalyses N-myristoylation, the best-understood protein acylation process, is the subject of one full article. Fibronectin is a multifunctional extracellular matrix protein with the 'most celebrated activity' of promoting cell adhesion. One review focuses on its cell-surface receptors, called integrins, with which the adhesion protein forms a recognition system that determines 'the information cells need to acquire and maintain the proper position, polarity, stage of differentiation and growth rate'. There is a sentence in this article which, when read in isolation, gives a wierd meaning: 'The cDNA sequence is human and the protein sequence bovine.' (!) The molecular organization and function of the complement system, with its 20 plasma proteins that play an important role in host defence in infection and inflammation, have been comprehensively covered. The glycoproteins of the zona pellucida, the thick, transparent coat of mammalian eggs, and their participation in the process of fertilization have been described with illustrations.

A few selected topics related to the class II subregion of the human major histocompatibility complex, its genetic organization, polymorphism of the genes and proteins, and regulation of their expression have been covered in another article.

One article focuses on insights into the evolution and expression of lens crystallins. The lens crystallins provide an excellent system for studies on tissue-specific gene expression. The crystallins are abundant soluble proteins and constitute 20–60% of the wet weight of the tissue in which they are expressed. The last decade has seen much development in the molecular and cellular biology of intermediate filaments, the prominent components of the cytoskeleton and nuclear envelope, the structural proteins of which are involved in specialized functions related to differentiation and signal transduction. A review brings this rather special, fast developing subject to the attention of biochemists. Another less-known area, that of peptide toxins from venomous snails, has been presented in sufficient detail.

An article on viral proteinases explains the importance of proteinases in the processing of precursor polypeptides, which yields active products necessary in the viral replication cycle, and also the usefulness of these studies in the development of specific protein inhibitors as antiviral agents. The synthesis of peptides and proteins has been of continual interest in biochemical studies and the current interest in biologically active peptides seems to have prompted inclusion of a review to 'provide an interpretive overview of synthetic methods that a non-specialist can use as a guide, but that an expert will not find seriously deficient'. The review justifies the need for chemical approaches in future biological studies.

Work in the last five years has revealed modes of attachment of membrane-bound proteins through non-protein anchors other than through acylation. A glycosylphosphatidylinositol linkage to the carboxy-terminus through ethanolamine has been shown. Treatment with bacterial phospholipase C, specific for cleaving phosphatidylinositol, released from the membrane surface alkaline phosphatase, acetylcholine esterase and 5'-nucleotidase, which then could not rebind. The finding of this mode of attachment resolved the enigma that proteins with no obvious hydrophobic sequences (needed for membrane localization) can be attached to membranes and function as localized molecules; examples are the Thy-1 antigen and the *Trypanosoma* variant surface glycoproteins. A review on this topic deals with structural and biosynthetic aspects of this anchor mechanism and offers useful guides for further research.

The article on growth factor receptor tyrosine kinases is an excellent overview of this rapidly expanding field that includes growth factors, protein kinases, transcriptional activation of specific genes, and oncogenes and cellular transformation. Interestingly the seven receptor tyrosine kinases studied have a common architecture and membrane topology—an extracellular, large ligand-binding domain and a large, intracellular catalytic domain (about 500 amino acids) linked by a single hydrophobic transmembrane segment. It is expected that exciting information will be forthcoming on how the ligand binding to the receptor generates the signal and how the signal is transmitted through the membrane and transduced as ATP-dependent phosphorylation of protein tyrosine (845 in human EGF-R). Another article introduces cachectin, a macrophage-derived protein that functions as an essential mediator of the inflammatory response and is capable of evoking a 'shock' state and inducing anorexia and wasting and haemorrhagic necrosis of tumours.

Several compounds that inhibit essential biosynthetic reactions are useful as herbicides and those that affect 'photosynthesis and essential amino acid biosynthesis, unique to plants and microbes,' are more appropriate and likely to be less toxic to animals. There is a review of the current understanding of plant enzymes and the genes involved in the biosynthesis of amino acids, and the possibility of using herbicides targeted at them. Although studied from the begining of biochemistry, regulation of carbohydrate metabolism is still an actively studied field. Hormonal regulation of hepatic gluconeogenesis and glycolysis is reviewed, including studies on pyruvate kinase, 1- and 2-kinases of fructose 6-phosphate, and phosphatases of 1,6- and 2,6-biphosphates of fructose.

Some general subjects have been included. One of these is an article on bacterial electron transport chains, by no means a new area but one that is now studied using genetic and molecular-biological approaches. This article describes bacterial aerobic respiratory chains and cytochromes, extensively covering the systems in *E. coli* and *P. denitrificans*. While there is some survey of peculiar electron transfer components, the omission of Indian work on *Agrobacterium tumefaciens* is glaring. The latter showed the presence of ubiquinone with 10 isoprene units in the side chain and antimycin-sensitive oxidation of NADH, both unusual for bacteria. Polyphosphates occur widely, and although a universal role for these has not been demonstrated, one of their roles seems to be to act as phosphorylating agents. This is described in one review. Another article is devoted to carnitine, its enzymology, and its impact on short-term metabolic control. The enzyme dopamine-β-hydroxylase, which catalyses the conversion of dopamine to the neurotransmitter and hormone norepinephrine, present in chromaffin granules of adrenal medulla and synaptic vesicles of sympathetic nervous system, is the subject of a review that attempts to integrate advances in studies on the nature of the enzyme with its function in the granules. A review with the novel title 'Glycobiology' discusses the current theories concerning the role of N-linked oligosaccharides in protein-specific biological activity. Blood clotting has attracted attention for several years, and the recent 'dramatic advances on sequences of cDNA coding for factor VIII, factor V, tissue factor and prothrombin' seem to have prompted a review on 'current perceptions of the multicomponent complexes associated with blood clotting with particular emphasis on the structural and functional aspects of the membrane-related cofactors'.

There has been a large growth in the literature of biochemistry and related areas and the trend is for more growth. Each year, with a selected few topics for these reviews, the *Annual Review of Biochemistry* provides a comprehensive presentation of the advancing frontiers. This year's volume, with 30 pages and possibly about 10,000 references contributed by about that many authors (list running to 82 pages), confirms the continuing expansion of interest in this subject. It is to the credit of the editors that a blend of classical and molecular-biological approaches has been presented, and subjects that are not so popular, but are still important, have not been ignored. This reviewer would like to see more illustrations and summary tables of data and less descriptive matter. With specialization for each worker becoming narrow, even these reviews have tended to become too large to comprehend, and alternatives are awaited.

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Annual Review of Cell Biology, vol. 4, 1988, pp. 754, (eds.) G. E. Palade, B. M. Alberts and J. A. Spudich, (Published by Annual Reviews Inc., 4139 El Camino way, Palo Alto, CA 94306, USA). Price: US$39.

The field of cell biology is fast expanding in many directions and encompasses all aspects of organization and function of living cells. The editors have rightly pointed out that 'it is not easy to generate a well-balanced Annual Review'. This volume has 24 articles covering widely different topics that look like a collection of those available. Realizing that it is not easy to give any themes by combining some of these articles, the reviewer decided to give an overview of these in the same sequence. The first article describes recent crystal studies of DNA for the comprehension of biologists, and includes the excitement of left-handed DNA, partly generated from Indian work. The current knowledge of adaptation to the environment in *E. coli* with respect to the outer membrane proteins (omp), which act as pores, has been reviewed. Not many would be familiar with 'growth cones, the specialized motile structures at the elongating tip of neurons', although the subject has been well reviewed during the eighties. The review here focuses on work during 1985–88, especially 11 papers of about 60 quoted, without any pretension of impartiality. Hair cells, the epithelial receptor cells that carry out transduction of mechanical stimuli into electrical responses in the auditory system, are now amenable to experiment, and a review on the topic describes how this difficult area is now yielding information. Regulation and physiological functions of cell-associated plasminogen activation that leads to formation of a serine proteinase, plasmin, are reviewed. The finding that DNA sequences lying a considerable distance upstream of transcriptional start sites are involved in transcriptional regulation has led to the concept of 'enhancers' distinct from 'promoters' and of their general occurrence as cellular transcription control elements. A description of their modular organization, their positive, negative and inducible regulation, and the involvement of protein–protein interactions have been summarized in a crisp article that is worth reading.

Mitochondrial membrane potential is known to be derived from respiratory chain electron transport and is considered to drive the 'proton turbine' of F_0 F_1-ATPase to synthesize ATP. New data with the use of a unique probe, rhodamine 123, a highly specific fluorescent dye, are discussed, which now indicate that such potentials are indeed obtained in living cells. Using inherent self-assembly properties of the components, living cells generate ordered supramolecular arrays. This fascinating problem of cell biology has been analysed with particular reference to the extracellular matrix.

Flowering plants have a requirement of outcrossing and those that are hermaphroditic also possess a mechanism for preventing self-fertilization. This mechanism is known as self-incompatibility, and is controlled by the S-gene. An article describes recent work, using recombinant DNA technology, on this subject.

Proteoglycans are proteins that carry sulphated carbohydrate components, glycosaminoglycan, with functions in cellular processes as diverse as matrix assembly, cell adhesion, differentiation and proliferation. Studies on their structure and biology are brought together in one review.

The endoplasmic reticulum (ER), an extensive membrane-bound organelle in the cytoplasm, is the site of synthesis of proteins. The sorting of proteins that are resident in the ER, such as those that synthesize some lipids and oligosaccharides, microsomal redox systems, and enzymes involved in correct protein folding, of those exported outside the cell, and of those targeted to lysosomes, mitochondria, plasma membrane and the cytosol is a very active field now, and this subject is covered by a comprehensive review. In another article the focus is on mitochondrial biogenesis, dealing essentially with genes of mitochondrial DNA and the integration of nuclear and mitochondrially synthesized proteins in the membrane structures of this organelle. Following this, another review deals exclusively with the functional organization of the nuclear envelope, especially important in eukaryotic cells to separate, 'spatially and temporally', the transcription and maturation of mRNA in the nucleus from its translation in the cytoplasm. The envelope is now known to consist of a double membrane and pore complexes with a lining of fibrous protein meshwork (lamina), which together regulate nucleocytoplasmic transport, a selective, vectorial process. Another article summarizes work on the mechanism of intervention of the embryonic microenvironment in establishing phenotypic diversity during neural crest ontogeny. This is an example of a classical anatomical descriptive approach to a specialized process like emergence of the peripheral nervous system.

A great deal of interest has been generated recently on membrane receptors and signal processing. Beta-adrenergic receptor, adenylyl cyclase G-proteins and the desensitization phenomenon have been discussed in a well-organized review. The process of conjugation in *Saccharomyces cerevisiae* is the basis of another article, with a view to understand the genes involved and their products. Under the interesting title 'From egg to epithelium', the anatomical changes associated with emergent epithelial cells, and the development of cytocortex and cytoskeleton as well as intercellular contacts, junctions and matrices, and intracellular organelles, are described. It is now possible to narrow down the areas on the plasma membrane responsible for the adhesion of cells to the underlying substrate in tissue culture, called adhesion plaques or focal contacts. Work on this less-known subject is summarized and this should stimulate further work. The kinetochore, the structure responsible for the attachment of mitotic chromosomes to the spindle and for their correct segregation, and its interaction with microtubules are the subject of another review.

The article 'New collagens' is likely to evoke much response, given the long-standing interest in work on collagens in India. Collagens, the structural proteins found in extracellular space, are now recognized to be a group of twelve distinct proteins encoded by different genes. Their major structural feature is the triple-helical conformation.

Lipids in membranes play the dual role of providing a permeability barrier and regulating the activity of membrane-associated enzymes. The biosynthesis of lipids and their transmembrane and intermembrane mobility have been reviewed. One omission in this review seems to be that of ubiquinone (coenzyme Q), and some earlier work from our laboratory and later work from Hackenbrock on its mobility could well have found a place. Post-translational modification of proteins through acylation of side-chains of amino acids by fatty acids is now recognized to be of wide occurrence. A review is devoted to the process of fatty acylation of proteins, which is an example how a single biosynthetic process can affect a wide variety of cellular processes.

Eukaryotic chemotaxis is an intriguing biological phenomenon. A review, covering the basic features of this process in biochemical terms, includes receptors, signal transduction, phospholipid-induced messengers, cyclic nucleotides, motility factors, G-proteins and cytoskeletal structures. Lastly, there is a review covering the structure and utilization of isotypes of tubulin, the subunit protein of microtubules. Tubulin is a heterodimer of two 50 kDa proteins that self-associate into hollow cylinders.

This volume will be highly useful to workers in biochemistry, biophysics, and cell and molecular biology. It is worth spending time reading these reviews. The awesome pace at which progress is being made in these areas changes the styles and capabilities in cell biology research. These reviews convey a feel of these changes. It is no wonder that Annual Reviews are the best-read and best-quoted work.

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ANNOUNCEMENTS

TWENTYSECOND ANNUAL MEETINGS OF THE NUTRITION SOCIETY OF INDIA

The meetings will be hosted by the Trivandrum Chapter on 10 and 11 November 1989 at Trivandrum. There will be symposia and special lectures on the following subjects:

Symposium I: Women — Health and nutrition
Symposium II: Iodine in human nutrition
Special Lectures: (1) Energy metabolism in Indians
(2) Chronic calcific pancreatitis

There will also be sessions for presentation of free communication papers. Abstracts, of not more than 200 words, in duplicate, must be sent before 31 August 1989 to Dr C. R. Soman, Secretary, XXII Annual Meeting of the NSI, Department of Applied Nutrition, Medical College, Trivandrum 695 011.

Awards for research papers

Cash awards for the best papers in Community Nutrition and Experimental Nutrition will be presented. Four copies of the paper and abstract must be sent before 1 August 1989 to the Joint Secretary, Nutrition Society of India, National Institute of Nutrition, P.O. Jamai Osmania, Hyderabad 500 007. For details of conditions for the awards, contact the Joint Secretary, NSI.

INSA BURSARY GRANT SCHEME

The Indian National Science Academy invites applications for Bursary grant up to Rs. 20,000. This is a limited *one-time* supplementary support to individual scientists for carrying out research effectively. The grant under the scheme is available for purchase or fabrication of equipment, purchase of components or accessories, or for purchase of some rare chemicals (which are not available in the laboratory), and other items concerned with research such as for defrayal of computational charges. The grant is also available for organizing small group discussions on important aspects of research and development and for field work. The Bursary grant is not available for attending meetings overseas or for appointments of secretarial assistance.

The application form for Bursary grant may be obtained from the Project Officer (Basic Sciences), Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002.

COMMENT

COLD FUSION: IS THERE A SOLID STATE EFFECT?

RECENTLY Fleischmann and Pons[1] have reported experimental observation of an 'excess' heat production accompanied by emissions of gamma rays and neutrons when isotopic hydrogen (D_2) was infused electrolytically into a palladium cathode to near saturation level ($\sim 1 D_2/Pd$). Independently, Jones and coworkers[2], using a somewhat similar electrolytic cell and Ti as well as Pd as cathode, have reported details of observation of neutron emission peaking at ~2.4 MeV and significantly above the broad-spectrum background neutron count. Both groups have claimed this as evidence for a cold fusion occurring in the deuterated cathodes.

The diagnostic 2.4 MeV neutron emission is strongly suggestive of the allowed fusion reactions

$$\begin{aligned} d + d &\rightarrow {}^3\mathrm{He}(0.82\ \mathrm{MeV}) + n\,(2.45\ \mathrm{MeV}) \\ &\rightarrow t\,(1.01\ \mathrm{MeV}) + p\,(3.03\ \mathrm{MeV}) \end{aligned}$$

(with 1:1 branching ratio). The neutron flux detected by Jones *et al.* was, however, much smaller ($\sim 0.41\ \mathrm{s}^{-1}$ for a 3 g electrode of Ti) than that of Fleischmann and Pons ($\sim 4 \times 10^4\ \mathrm{s}^{-1}$ for a 15 g Pd electrode). Moreover, the latter was at least nine orders of magnitude much too small to be consistent with the observed rate of 'excess' heat production (~10 W per cm^3 of electrode volume). This large discrepancy indicates that (i) alternative aneutronic channels may be operative, (ii) the 'excess' heat may after all be of chemical origin, not altogether unexpected from the known chemistry of these transition metal hydrides, or (iii) the experimental results are simply wrong in terms of the heat balance reckoning or the neutron flux detection. Absence of contemporaneous control experiments in order to properly subtract for the varying cosmic ray neutron and gamma ray backgrounds, and inadequate ^{3}He and tritium assays are the obvious worrying points. While initially several laboratories around the world corroborated—some informally—the original findings of these two groups, negative results were soon reported, notably by major laboratories[3], questioning this claim of cold fusion. There is now no compelling reason to believe cold fusion, at least as a commercial source of energy[4].

Be that as it may, these experiments and the extensive discussion that followed have successfully focused attention on the single most important and as yet unresolved basic question, namely whether or not solid state effects can affect the fusion rate appreciably[5]. The question is not entirely devoid of practical importance, for, if the answer is affirmative, the solid state effects can be tuned to advantage. In the following we will address this problem in physical terms.

Consider first the simplest case of d–d fusion in a deuterium molecule D_2. The nuclear interaction causing fusion is strong but of short range ~2–3 fm ≪ bond length ~ closest distance of approach, classically $R_0 \simeq 0.7$ Å in the deuterium molecule D_2. The latter is essentially the internuclear separation (screening length), below which the charged nuclei are exposed to the bare Coulomb repulsion. The resulting so-called Coulomb barrier must be tunnelled through quantum-mechanically. The fusion rate is then limited by the barrier penetrability (Gamov) factor, which, for free d–d collision, as in the case of plasma fusion, is given by

$$\exp(-2\pi e^2 \sqrt{M}/\hbar \sqrt{2E}).$$

This has a sensitive (exponential) dependence on the centre-of-mass energy E, which in turn determines the tunnelling distance ($\simeq R_0$ under the barrier). For the case of molecular fusion in D_2, E is effectively $\sim e^2/R_0$. The sensitive dependence on the tunnelling distance R_0 is, of course, well demonstrated by the known muon-catalysed fusion in muomolecular deuterium ion $(dd\mu)^+$. Here the electron is replaced by a negative muon (heavy electron), which is about 200 times as massive. This leads to a contraction of the Bohr radius (and, therefore, of the screening length R_0) by the same factor, leading to a fusion time in D^+ of $\sim 10^{-11}$ s, which is an 85 orders of magnitude enhancement over the fusion rate of $\sim 10^{-74}$ fusions per deuteron pair per second for the electronic deuterium molecular ion $(dde)^+$. (Indeed, there has been a suggestion that cosmic muons may have catalysed the reported fusion in deuterated palladium/titanium. However, realistic estimates based on the observed muon flux, their tendency to get stopped and captured by high-Z nuclei, and their short lifetime of $\sim 2.2\ \mu$s rule this out.) From the Gamov factor one can readily see that to get the inferred fusion rate of $\sim 10^{-23}$ per d–d pair per second, all that is necessary is to make the electronic effective mass just about 6 times its bare mass, thus reducing the bond length from 0.7 Å to 0.1 Å and effectively increasing E from 20 eV to $\sim 10^2$ eV. Naively one could hope for such an effective mass enhancement from the band structure and the Fermi-liquid renormalization effects in deuterated Pd/Ti. Transition metals do have large effective masses and in heavy fermion systems $m_{\mathrm{eff}}/m \sim 10^2$ is common. When a D_2 molecule is introduced into metallic palladium, the covalent bond in D_2, which may be regarded as a highly nonlinear screening of d–d Coulomb repulsion by the bound valence electrons, is expected to be replaced by a metallized bond (electron gas screening), giving D^+ ions. For a simple electron gas model of the host metal, the condition for this ionization is

$$K_c \hbar^2 / Zme^2 > \sqrt{2},$$

where Z (= 1) is the charge on D^+, and

$$K_c^2 = 4\left(\frac{me^2}{\hbar^2}\right)\left(\frac{3n}{\pi}\right)^{1/3},$$

the reciprocal of Thomas–Fermi screening length squared for the electron concentration n. For deuterated Pd/Ti this condition is satisfied. It is clear that the screening length and, therefore, the closest d–d separation for free encounter can be reduced by enhancing the electron mass effectively by the above-mentioned solid state effects. Unfortunately, the solid-state effective mass and the associated screening is meaningful only for electronic motion on the length scale $> a$ (lattice constant) $\simeq 2$–3 Å. For the local motion on the length scale ~ 0.1 Å $\ll a$, it is the bare inertial mass that is relevant. Since the metallic screening length $1/K_s$ is typically $\sim a >$ covalent bond length R_0, metallization of the D_2 bond as in deuterated Pd/Ti is detrimental to fusion. The above electron gas-based arguments may not apply realistically to the actual lattice-bound deuterons that occupy octahedral (tetrahedral) sites in the Pd (Ti) host. There is, however, a global argument[6] that rules out formation of compact d–d pairs in Pd/Ti host.

The point of the argument is that when the d–d distance is ≪ lattice spacing (i.e. both deuterons occupy the octahedral/tetrahedral sites in the host Pd/Ti), the intimate pair should act as an alpha particle (helium nucleus), except for the large positive Coulomb energy of d–d repulsion. Now, since helium is known to spontaneously desorb from the host Pd/Ti, the assumed compact d–d pairs can only do worse energetically and will simply not form. This is essentially the 'helium trick' recently formalized by Leggett and Baym[7] in the Born–Oppenheimer approximation.

Finally, we turn to a nonelectronic and nonequilibrium solid state effect that seems plausible. First let us note that the electrolytic infusion is not essential to 'cold fusion'. Similar effects have been reported by the Italian group[8] who deuterated titanium under pressure/temperature instead, and neutron bursts were detected during absorption and desorption due to change of thermodynamic conditions. Thus what is essential is a condition of nonequilibrium. Well-equilibrated deuterated samples do not emit neutrons. After all deuterated compounds such as d_4-ammonium halides with comparable d–d distances do not emit neutrons. Under the nonequilibrium conditions it seems possible to pump energy associated with motion/relaxation of macroscopic defects into a microscopic degree of freedom, e.g. accelerate deuterons to kinetic energy of $\sim 10^2$–10^3 eV, far in excess of the equipartition value. An example would be a propagating crack in embrittled Pd/Ti with deuterons trapped in the tip. (Deuterons, like water molecules, are expected to lubricate crack propagation.) Such nonthermodynamic defects are 'intelligent heat engines' driven by extended random stress fields due to conditions far from equilibrium, and funnel energy into the microscopic propagating tip. Neutron emission accompanying fracture in LiD crystals is understood in these terms[9]. It should be interesting to study neutron emission from a vibrating reed mode from deuterated Pd/Ti (Gorsky effect-induced). It should be remarked here that the Born–Oppenheimer potential for deuterons in metallic Pd/Ti may have relatively large three-body and many-body components in addition to the two-body component. On the one hand this makes quantum tunnelling multidimensional with preferred reaction coordinates having low barrier, and on the other it helps pumping of energy into a certain degree of freedom as in a driven system of nonlinearly coupled modes. This calls for further study.

11 July 1989

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CORRESPONDENCE

Science and audit

Since the objective of the editor of *Current Science* is to bring about better understanding between scientists and auditors, I thought fit to respond to the article 'Are scientists profligate?' (*Curr. Sci.*, **58**, 673). It must be clarified that audit of scientific departments has been unified and undertaken in a systematic manner since April 1986. Earlier, departments like Atomic Energy (DAE) and Space (DOS) were protected from public glare for the reason that new technologies needed to take root and scientific advancement was more important than audit objections. More than 35 years have elapsed in the case of DAE and about 15 years in the case of DOS and so these have been subjected to audit now.

In a democracy, it is axiomatic that a constitutional authority should audit governmental activities funded with public money. The people have delegated the responsibility to this authority in good faith.

The author of the article seems to be confused between a single scientist and a department. He has also not discriminated between smaller institutions and monoliths like DAE. It is my conviction that scientists are doing their best, but when a few of them try to build empires all the ills of a big organization creep in.

When we are talking about delays, we are not talking about delays of one year or so. The audit reports highlight delays of 10–15 years in the case of atomic power plants and delays of up to 9 years in installing imported equipment in about 90 cases in various CSIR laboratories. The latest audit report has also highlighted a case where land acquired 20 years ago has not been put to use in Maharashtra. These few examples should show that it is not small delays or stray cases that attract audit comment.

Autonomy is not anathema to audit. In fact it is for the executives and scientist-administrators to decide what form of organization they need to further scientific achievements. Unless scientists themselves redraft the rules to make them helpful to the progress of science they will not be able to get out of the present quagmire. Pray, why has not CSIR done this in the last 50 years?

The author has mentioned that he would welcome performance audit by peers. Performance audit is done with reference to benchmarks indicated by the scientists themselves in their project reports etc. When failures and shortfalls of serious magnitude are brought out they do not analyse the reasons and take corrective action. We live in an age of multidisciplinary approaches, which should be true even for research programmes. Scientists can extend the idea and include auditors in performance evaluation and avoid the dangers of inbreeding.

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ARTICLES

CARRIER DENSITY FLUCTUATIONS IN SINGLE-INJECTION SOLID-STATE DIODES WITH TRAPS LYING BELOW THE FERMI LEVEL

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ABSTRACT

The effects of space charge and trapping on low-frequency noise in single-injection solid-state diodes with traps lying below the Fermi level have been studied in the complete range of current–voltage characteristic. It is shown that the complete noise spectrum may be divided into four noise regimes and the low-frequency noise at high injection level of current is very much dependent on the injection level of current.

INTRODUCTION

IN single-injection devices, current flow is dominated by the space charge, which gives nonlinear effects on current–voltage characteristics and noise spectrum[1-4]. Carrier density fluctuations in an insulator give low-frequency noise in injection diodes[1]. The presence of trapping states changes the current–voltage characteristic by the trapping effect and the noise spectrum is modulated under the influence of trapping.

CURRENT INJECTION

According to the current injection theory[2-4], an insulator containing traps lying below the Fermi level may be divided into three regions by two imaginary transition planes x_1 and x_2, which shift towards the anode with increasing injection level of current. The regional approximation scheme for the problem is shown in figure 1.

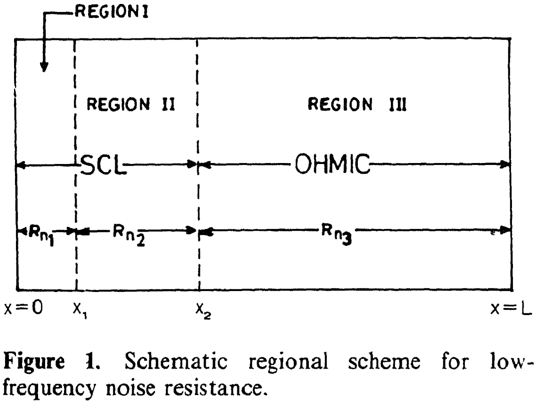

Figure 1. Schematic regional scheme for low-frequency noise resistance.

The general equations are obtained as[3]

region I: $(0 \leqslant x \leqslant x_1)$: $n_0 \ll n(x)$, $p_{t,0} \ll n(x)$

$$J = e\,\mu\,n(x)\,E(x),\ \frac{\varepsilon}{e}\frac{\mathrm{d}E}{\mathrm{d}x} = n(x), \tag{1}$$

region II: $(x_1 \leqslant x \leqslant x_2)$: $n_0 \leqslant n(x) \leqslant p_{t,0}$,

$$J = e\,\mu\,n(x)\,E(x),\ \frac{\varepsilon}{e}\frac{\mathrm{d}E}{\mathrm{d}x} = p_{t,0}, \tag{2}$$

region III: $(x_2 \leqslant x \leqslant L)$: $n(x) - n_0 \ll n_0$,

$$J = e\,\mu\,n_0 E,\ \frac{\varepsilon}{e}\frac{\mathrm{d}E}{\mathrm{d}x} = 0, \tag{3}$$

where J is the current density, μ the mobility of current carriers, ε the permittivity of the insulator, n_0 the thermally generated free carriers, $n(x)$ the concentration of carriers at distance x, L the device length, and $p_{t,0}$ the thermal equilibrium value of the concentration of traps not occupied by electrons.

The transition planes connecting the separate regions are defined as[3]

$$n(x_1) = p_{t,0},\ n(x_2) = n_0, \tag{4}$$

where

$$x_1 = \frac{\varepsilon J}{2A^2 e^2 \mu n_0^2},\ x_2 = \frac{\varepsilon J}{A e^2 \mu n_0^2},\ A = \frac{p_{t,0}}{n_0}. \tag{5}$$

The detailed theory of current injection in insulator diodes with traps lying below the Fermi level is described elsewhere[2-4].

LOW-FREQUENCY NOISE

The low-frequency noise characteristic starts from the pure Ohm's regime and the last regime is obtained by space charge conduction[2-4]. Adopting usual procedures[1,2], equations (1)–(5) are used to derive the expression for noise resistances in three regions as given below:

$$\text{region I:}\quad R_{n_1}=\frac{\varepsilon^2 J^2 S}{288\,k\,T\,A^8\,e^7\,\mu^4\,n_0^8}, \qquad (6)$$

$$\text{region II:}\quad R_{n_2}=\frac{\varepsilon^2 J^5 S}{32\,k\,T\,A^4\,e^7\,\mu^4\,n_0^8}\left[1+\frac{1}{16\,A^4}-\frac{1}{2\,A^2}\right], \qquad (7)$$

$$\text{region III:}\quad R_{n_3}=\frac{J^2}{2\,kT\,e^2\,\mu^2\,n_0^3}\left[L-\frac{\varepsilon J}{A\,e^2\,\mu\,n_0^2}\right]^2, \qquad (8)$$

where S is cross-sectional area, k Boltzmann's constant, T the lattice temperature, and A is as given in (5). The total noise resistance of the diode is given by

$$R_n=R_{n_1}+R_{n_2}+R_{n_3}. \qquad (9)$$

At very low injection level of current, only region III contributes to the low-frequency noise of the diode. This is the true Ohm's regime of low-frequency noise characteristic, whose noise resistance is obtained from (8) as

$$R_t=\frac{J^2\,L^2}{2\,k\,T\,e^2\,\mu^2\,n_0^3}, \qquad (10)$$

where region III extends from cathode to anode and the transition plane x_2 is close to the cathode.

The expression for low-frequency noise in the ohmic regime is considered for the injection level at which all three regions are present in the insulator. The noise resistance for this regime is derived from equations (6)–(8):

$$R_0=\frac{J^2}{2\,k\,T\,e^2\,\mu^2\,n_0^3}\left[\frac{\varepsilon^2 J^3 S}{144\,A^8\,e^5\,\mu^2\,n_0^5}+\frac{\varepsilon^2 J^3 S}{16\,A^4\,e^5\,\mu^2\,n_0^5}\times\left(1+\frac{1}{16\,A^4}-\frac{1}{2\,A^2}\right)+\left(L-\frac{\varepsilon\,J}{A\,e^2\,n_0^2}\right)^2\right], \qquad (11)$$

which is a complex relation depending on the injection level and physical parameters of the diode.

Regions I and II are considered to obtain the noise resistance of the diode at medium injection level of current because the injected current carriers are distributed over all parts of the diode and region III is not present in the insulator. Equations (6) and (7) give

$$R_m=R_{n_1}+R_{n_2}=\frac{\varepsilon^2 J^5 S}{32\,k\,T\,A^4\,e^7\,\mu^4\,n_0^8}\left[1-\frac{1}{2\,A^2}+\frac{25}{144\,A^4}\right], \qquad (12)$$

where the different sets of noise resistances are obtained for different values of the parameter A.

At high injection level of current, the low-frequency noise is generated in only region I. The noise resistance of the diode in this regime is derived from equation (6):

$$R_h=\frac{\varepsilon^2 J^5 S}{288\,k\,T\,A^8\,e^7\,\mu^4\,n_0^8}, \qquad (13)$$

which is strongly dependent on the injection level of current. The space charge conduction mechanism is dominant at high injection level.

DISCUSSION

The complete low-frequency noise characteristic is divided into four noise regimes. The characteristic starts from very low injection level of current, at which the noise resistance is obtained only by the ohmic contributions. All the three regions are present in the insulator in the ohmic regime; therefore the noise characteristic is obtained by the space charge, trapping and ohmic contributions. The trapping effect is dominant in the low-frequency noise characteristic at medium injection level of current. The parameter A gives different sets of noise resistances for different insulating materials. The low-frequency noise of the diode at high injection level of current is controlled by space charge and the trapping and ohmic effects are negligibly small.

27 August 1988; Revised 23 January 1989

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DISCOVERY OF PRE-TRILOBITE SMALL SHELLY FOSSILS AND THE POSITION OF PRECAMBRIAN–CAMBRIAN BOUNDARY IN TETHYAN SEQUENCE OF NORTHWESTERN KASHMIR

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ABSTRACT

The present paper records for the first time a pre-trilobite small shelly fossil assemblage comprising *Protohertzina* Missarzhevsky, cf. *Taliella* Azmi, *Olivooides multisulcatus* Qian, *Gaparella porosa* Missarzhevsky, and a new type of sponge spicule from greyish black slate of the lower part of Lolab Formation in the northwestern part of the Kashmir basin. This assemblage of small shelly fossils occurs below the oldest trilobite (*Redlichia noetlingi*) and trace fossil (*Rusophycus–Cruziana*) zone, which clearly indicates that the boundary between Precambrian and Cambrian lies within the lower part of Lolab Formation. The faunal assemblage approximately correlates with the Precambrian–Cambrian boundary fauna of the Chert–Phosphorite Member of the Lower Tal Formation of Lesser Himalaya and other regions such as south China, Siberian Platform, Mongolia, Kazakhstan and western Canada.

INTRODUCTION

An assemblage of pre-trilobite small shelly fossils (SSF) of Precambrian–Cambrian boundary age was reported by the author for the first time from the Tethyan sequence of the northwestern part of the Kashmir basin[1]. The initial stratigraphy of Kashmir is based mainly on the work of Wadia[2], Reed[3] and Kobayashi[4]. Since 1965 further intensive and detailed biostratigraphical work has been carried out by others[5-18]. These studies were primarily based on trilobite and trace-fossil assemblages which allowed a detailed biostratigraphical subdivision of sedimentary sequence (figure 1) and its correlation with other fossiliferous sequences of similar age elsewhere in the world[14].

The earliest body fossil recorded is the *Redlichia* fauna in association with obolellid, lingulellid and paterinid brachiopods of Botomian Stage[8] in the upper part of Lolab Formation. The horizon containing abundant and diverse trace fossils, namely *Rusophycus, Cruziana, Monomorphichnus, Diplichnites, Kupwaria*, etc.[12], occurs just below the *Redlichia* zone. The upper part of Machhal and lower part of Lolab Formation also contain simple burrows, viz. *Planolites, Skolithos, Bergauria*, in association with Precambrian biota *Protospheridium, Granomarginata, Stictospheridium, Kildinella, Lophospheridium, Origmatospheridium, ?Saharadia* and some algal filaments[17, 18]. However, due to lack of any evidence of Tommotian/Meishucunian SSF, the Precambrian–Cambrian boundary was questionably placed at the contact of Machhal (= Merinag Formation) and Lolab Formation below the *Rusophycus–Cruziana* zone pending discovery of SSF[9]. Taking this point into consideration a vigorous search for SSF was started through extensive sampling in this region. The search ultimately resulted in the discovery of earliest small shelly fauna from greyish black slate in a small exposure of a *nala* east of village Parepur (figure 2) about 4 km north-east from Kalaruce village. This exposure, according to Shah[19], lies approximately 200 m below the earliest trace-fossil horizon in the immediate vicinity. The specimens were extracted using 10% acetic acid and were studied and photographed under a scanning electron microscope.

SMALL SHELLY FAUNA

The SSF assemblage comprises *Protohertzina* Missarzhevsky, cf. *Taliella* Azmi, *Olivooides multisulcatus* Qian, *Gaparella porosa* Missarzhevsky, and a new type of sponge spicule (figure 3, 1–5).

(i) *Protohertzina* Missarzhevsky, 1973 (figure 3, 1). Two incomplete specimens assignable to earliest conodont *Protohertzina*, bear a distinct median posterior keel with expanded base. Basal outline is nearly circular and the element is symmetrical. These specimens belong to *P. anabarica–P. unguliformis* group as they possess posteriolateral keels and

AGES			LITHOSTRATIGRAPHIC UNIT	KASHMIR (Modified after Shah & Sudan 1984)	KROL BELT LESSER HIMALAYA
ORDOVICIAN					
CAMBRIAN	UPPER				
		MAENTWROGIAN	TRAHAGAM FORMATION	*DAMESELLA* ZONE	
	MIDDLE	MENEVIAN	NUTNUS FORMATION	*BOLASPIDELLA* ZONE *ANOMOCARE-BAILIELLA* ZONE *TONKINELLA-ANOMOCARE* ZONE	
		SOLVAN		*SOLENOPLEURA-TONKINELLA* ZONE	
	LOWER	LENIAN	SAGIPURA FORMATION		TAL FORMATION
		ATDABANIAN	LOLAB FORMATION	*REDLICHIA NOETLING* ZONE *RUSOPHYCUS-CRUZIANA* ZONE	PHULCHATTI QZT. MEMBER CALCAREOUS MEMBER
		TOMMOTIAN		*PROTOHERTZINA-TALIELLA* ZONE	ARENACEOUS MEMBER ARGILLACEOUS MEMBER CHERT-PHOSPHORITE MEMBER
PRECAMBRIAN			MACHHAL FORMATION		KROL FORMATION

Figure 1. Lithostratigraphic and biostratigraphic correlation of the Cambrian succession of northwestern Kashmir and Krol–Tal succession of the Lesser Himalaya.

lateral furrows. It is known from Yodomian–Tommotian strata of Karatau Ridge, Kazakhstan and Siberian Platform, Meishucunian Stage in eastern Yunnan Province, and Lower Tal Phosphorite of Mussoorie Syncline, India.

(ii) cf. *Taliella* Azmi, 1987 (figure 3, 2). Three specimens showing a general resemblance with *Taliella* Azmi. It is a paired conodont cluster ornamented with faintly developed longitudinal ridges and tubercles. Three prominent ridges appear toward apical portion with terminal nodal spines. The difference between *Taliella* Azmi and the present form is that the former has prominent ridges and spines while the latter contains faint ridges with tubercles. According to Azmi (personal communication) the specimens in the collection fall within the morphological variation of *Taliella*. It is illustrated from Chert–Phosphorite Member of Lower Tal Formation, Maldeota, Garhwal Lesser Himalaya (see cover photograph of conference volume of Geology of Krol Basin in Garhwal, 12–14 October 1987).

(iii) *Olivooides multisulcatus* Qian, 1977 (figure 3, 4). Two specimens of simple spherical shape with granulose outer surface, comparable to *Olivooides multisulcatus* Qian. One of the specimens was broken during preparation, which showed the hollow and smooth character of the test. It is described from the lower part of Meishucunian Stage of China.

(iv) *Gaparella porosa* Missarzhevsky, 1981 (figure 3, 5). One specimen of nearly spherical shape, ornamented with hollow, short spines all over the body. It is known from the Lower Cambrian (Upper Atdabanian) of Russian and Siberian Platforms, Altai Sayan and Mongolia.

(v) Sponge spicule (figure 3, 3a, b). Four specimens have been found of this conspicuous spicule type. There are three main distally curved rays alternating with three minor rays at the proximal end and one acanthose ray. Suture line visible. Acanthose ray greatly expanded and the ray diameter increases toward the distal end, covered with more or less continuous ridges parallel to the axis of the ray, and small spines directed proximally. This type of spicule is so far not known from Precambrian–Cambrian

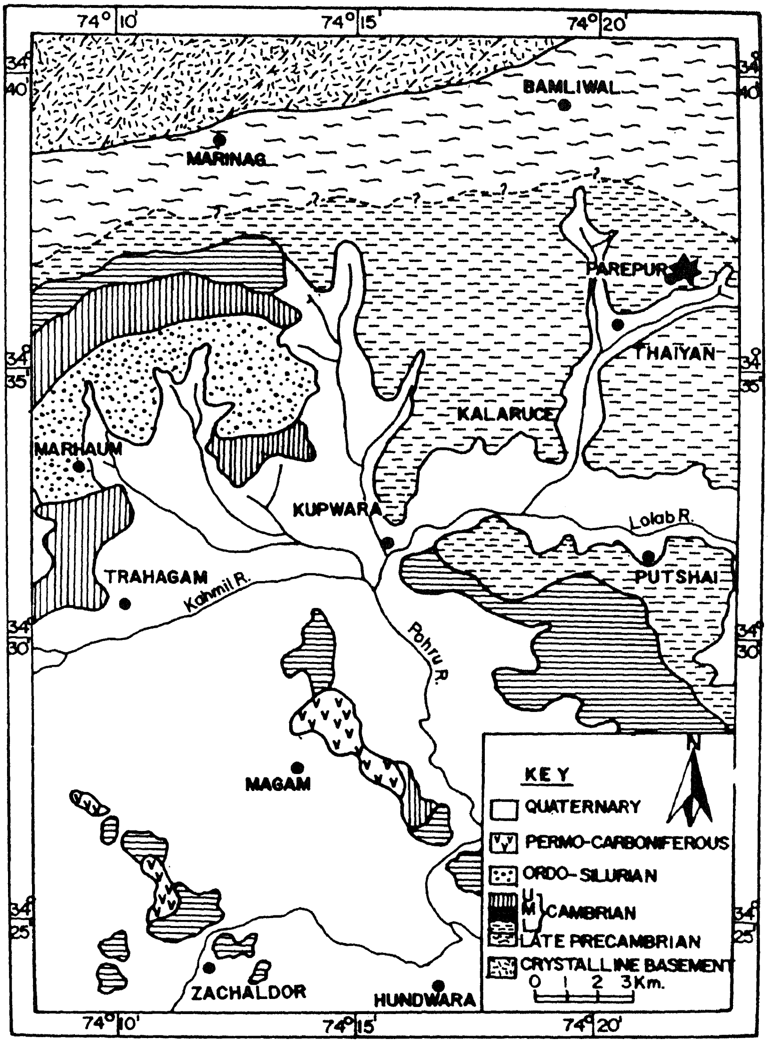

Figure 2. Geological map of northwestern Kashmir (after Shah, 1982), showing small shelly fossil locality.

boundary beds and is being published elsewhere with additional details.

All the illustrated specimens are catalogued, under Wadia Institute Repository numbers WIF/A 701 to 705.

CONCLUSION

The internationally accepted criteria for Precambrian–Cambrian boundary by IUGC/IGCP clearly indicates, 'The boundary should be placed as close as practicable to the lowest known appearance of diverse shelly fossils with a good potential for correlation (Cowie[20]). Although the assemblage discovered at the present stage is not rich it is broadly comparable and correlatable with the Precambrian–Cambrian boundary microfaunal assemblage of Krol–Tal sequence of Lesser Himalaya[21–24] (figure 1) and other world-wide localities such as China, Siberian Platform, Mongolia, Kazakhstan, northwestern Canada, etc. With the present discovery

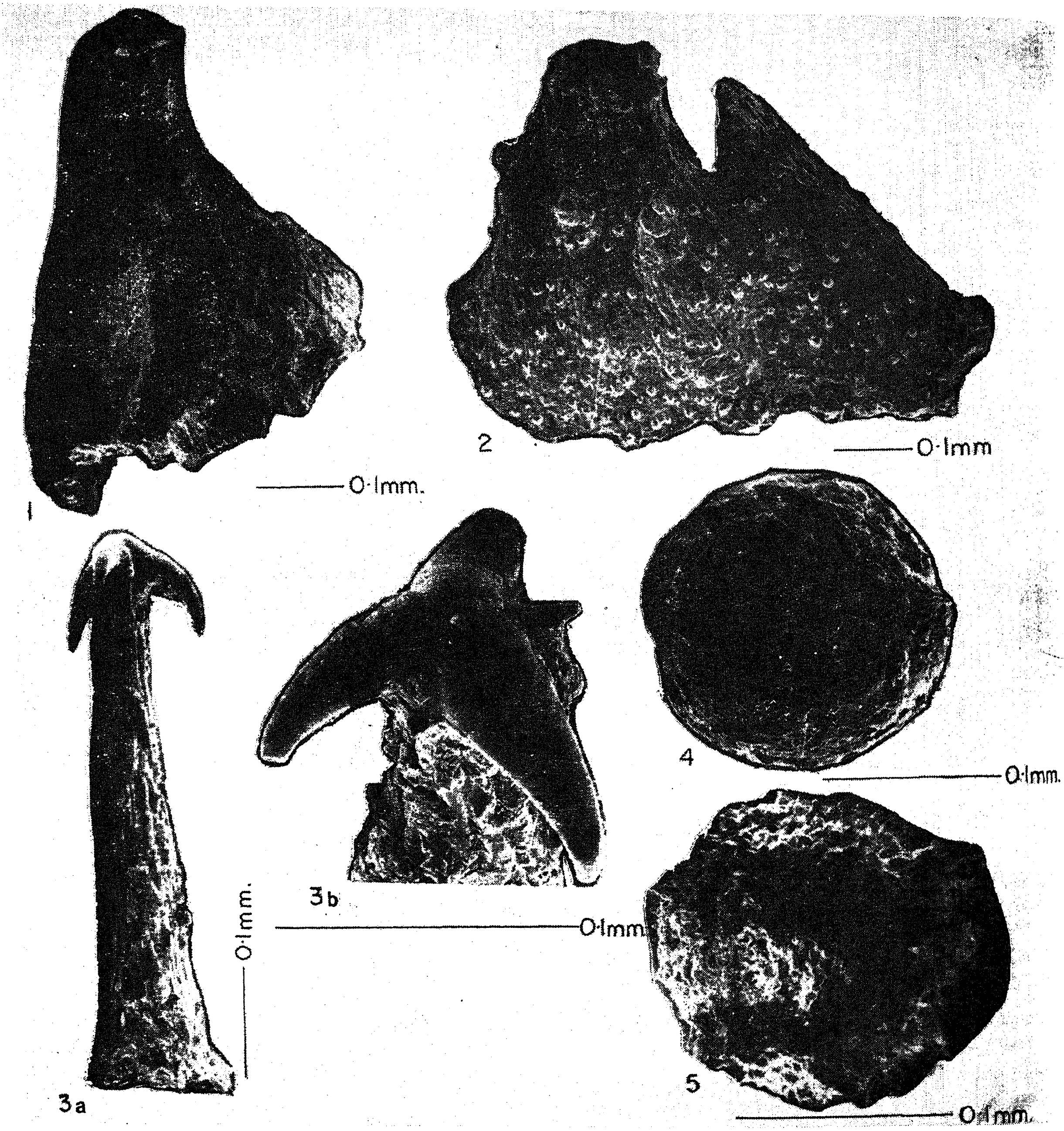

Figure 3. Precambrian–Cambrian small shelly fossils from northwestern Kashmir. **1,** *Protohertzina* Missarzhevsky (WIF/A 701); **2,** cf. *Taliella* Azmi (WIF/A 702); **3a,** sponge spicule (WIF/A 703); **3b,** enlargement of proximal part of 3a; **4,** *Olivooides multisulcatus* Qian (WIF/A 704); **5,** *Gaparella porosa* Missarzhevsky (WIF/A 705).

of pre-trilobite SSF it is now possible to identify small shelly fauna of the Tommotian/Meishucunian stage in the Tethyan realm of northwestern Kashmir and we can tentatively place the Precambrian–Cambrian boundary in the lower part of Lolab Formation. The precise position of the boundary, however, requires additional material to establish the SSF assemblage zone in this region.

ACKNOWLEDGEMENTS

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CALLUS CULTURING, ITS MAINTENANCE AND CYTOLOGICAL VARIATIONS IN *TRIGONELLA FOENUM-GRAECUM* L.

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ABSTRACT

Studies on callus culture and cytological behaviour were performed in *Trigonella foenum-graecum* L. cotyledonery segment and it was found to be the best explant for callus initiation as well as callus growth and maintenance in MS basal nutrient medium supplemented with NAA in combination with coconut water. Cytological studies revealed the abundance of heterogeneous cells with different chromosome numbers varying from 16 to 48. The present results indicate variable frequencies of ploidy level and anomalies in different subcultures.

INTRODUCTION

TRIGONELLA FOENUM-GRAECUM L. is traditionally cultivated for its spice-yielding seeds and leafy twigs. This plant is now gaining importance for its diosgenin content[1] and usefulness in the treatment of diabetes[2]. Being self-pollinated, the plant offers very limited scope for improvement through conventional breeding methods. Genetic variability, however, can be induced by the exploitation of cell and tissue culture techniques. The possibility of utilization of these techniques needs proper assessment, but only a few relevant references are available[1,3,4]. We therefore studied the behaviour of chromosomes in callus cells of *T. foenum-graecum*.

MATERIAL AND METHODS

Seeds of *T. foenum-graecum* (fenugreek) were surface-sterilized with 0.1% mercuric chloride for 5 min and then washed repeatedly with sterile distilled water. They were inoculated on Murashige and Skoog's (MS)[5] medium without any hormone for germination. The cultures were maintained in the dark for rapid germination at 22±2°C. Cotyledonary and hypocotyledonary segments of the aseptically grown seedlings were used as explants for callus initiation. Small segments of the explants (4–6 mm thick) were cultured in MS medium (pH 5.8–6.0) supplemented with naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin and coconut water (CW) for initiation and growth of callus. The cultures were maintained at 22±2°C and 55–60% RH under Philips fluorescent daylight tubes emitting $32 \times 10^8\ \mu\text{mol sec}^{-1}\ \text{m}^{-2}$ in a 10/14 h light/dark regime. Subcultures were carried out every 15 days.

For cytological observations cotyledon callus tissues were taken and fixed in acetic–ethanol (1:3). They were then slightly warmed in a 1% aceto-orcein-1 N HCl mixture (9:1), and kept for 1 h before washing in 45% acetic acid.

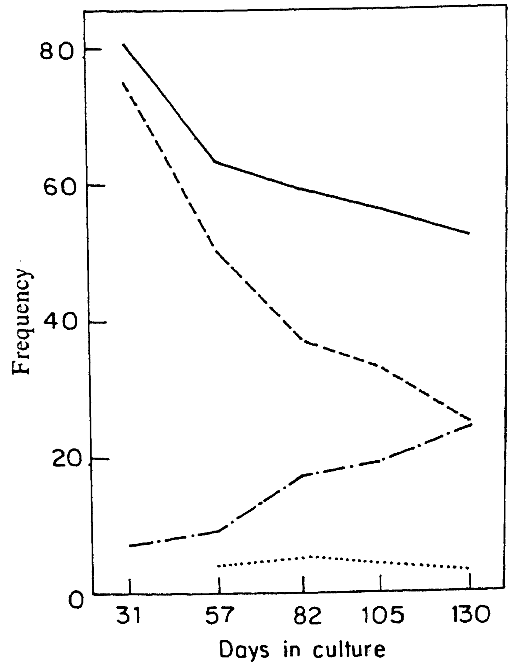
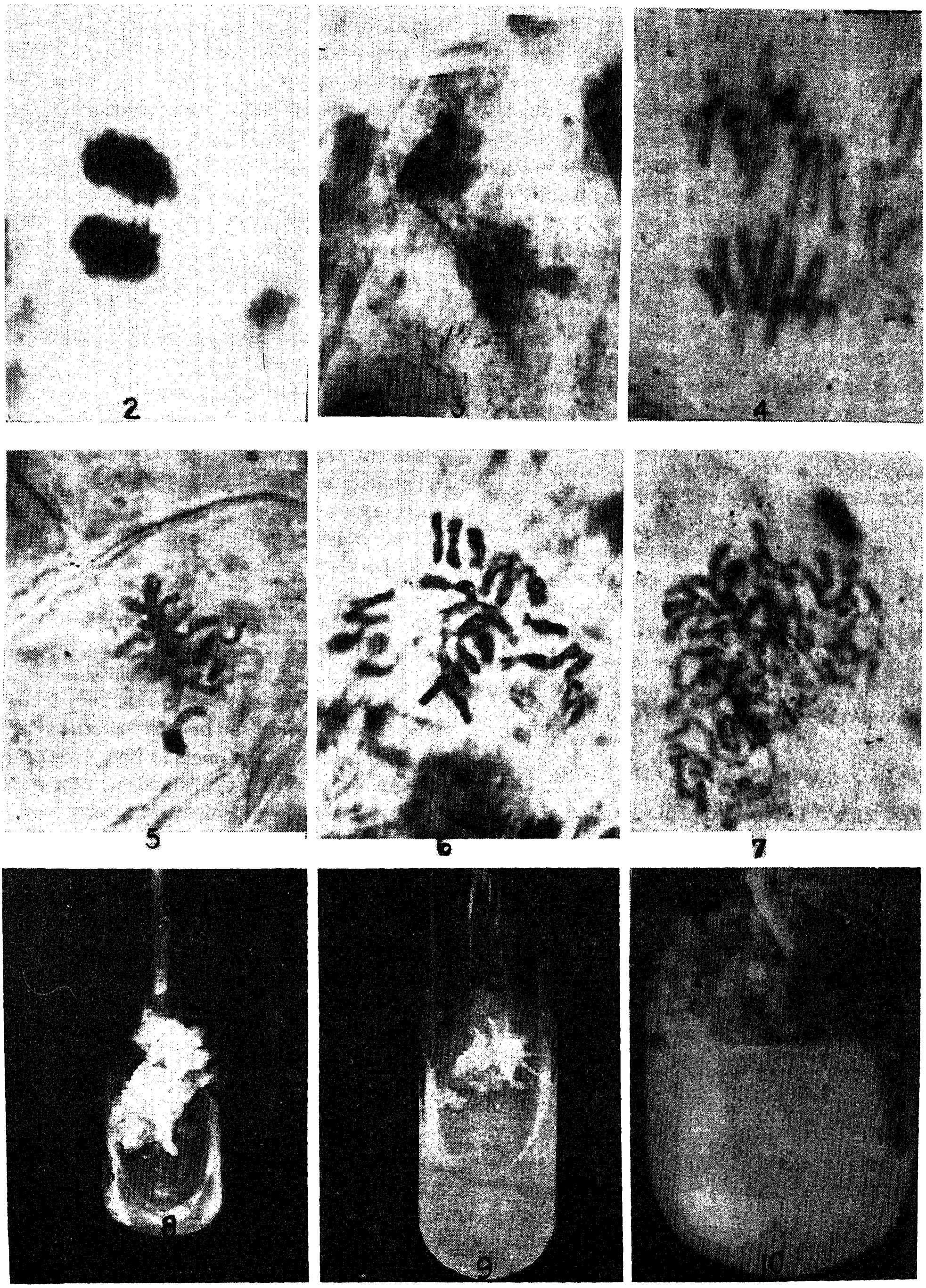


Figure 1. Frequency of diploid and polyploid cells in relation to age in culture (———— dividing cells, ---- diploid cells, —·—· tetraploid cells, ... higher-polyploid cells).



Figures 2–10. Cytology of cotyledon callus and callus growth and differentiation. **2,** Double bridge at anaphase. **3,** Bridge with laggard. **4,** Multipolar separation. **5,** Metaphase with $2n=16$. **6,** $4n=32$ at metaphase. **7,** Metaphase with hexaploid number. **8,** Callus from cotyledon. **9,** Root organogenesis. **10,** Callus with shoot buds.

RESULTS

Both cotyledonary and hypocotyledonary segments were swollen at the cut ends after two days. Callus tissue initiated on cotyledon segments within 5–6 days of inoculation but after 12–15 days in the case of hypocotyl. There was more callus proliferation from the cotyledon (figure 8). Among the growth hormones used NAA (2 mg/l) alone as well as in combination with 10% v/v CW resulted in better initiation and growth of the callus from the cotyledon. On the other hand neither 2,4-D nor kinetin alone could bring about any improvement in callus induction. Callus from cotyledon was soft, greenish white, friable and vigorously growing whereas callus from hypocotyl was milky to brownish white and compact. The appearance of whitish roots was noted on cotyledon callus at the third subculture, and at the end of this passage, both callus tissue and roots turned brown (figure 9). The callus in most cases turned brown to black and looked friable to granular, sticky and compact after two months (figure 10). There was no difference between cultures in callus initiation medium and in differentiating medium. The same growth hormone induced shoot bud formation and better callus development under identical conditions.

Cytological observations

Cytological studies on cotyledon callus revealed that mitotic index was very low in the initial cell population (table 1). The frequency of both diploid cells and total number of dividing cells decreased with age of the tissue but that of tetraploid and higher-polyploid cells increased (figure 1). The countable chromosome number in the somatic complement varied from a diploid number of 16 to 48 during growth and proliferation of cotyledon callus (figures 5–7). There was a high percentage (45%) of normal diploid cells ($2n = 16$) in cotyledon callus tissue at the first subculture. The percentages of triploid ($3n = 24$), tetraploid ($4n = 32$) and higher-polyploid ($6n = 48$) cells were 30, 28 and 13% at the 4th, 5th and 3rd subculture respectively.

Among the observed chromosomal aberrations, in cotyledon callus cells, bridges, laggards and multipolarity were common and were maximum at the 6th subculture (figures 2–4). These were less common in hypocotyl callus cells. Chromosomal bridges were the most common aberration (table 1).

DISCUSSION

The cotyledon explant showed better response than the hypocotyl, which clearly indicates that different parts of the same plant may show different responses to callus-inducing medium. In the present investigation, chromosomal instability was indicated by the occurrence of cells with polyploid chromosome numbers and different types of mitotic anomalies. Calli constitute an unorganized system where chromosomal instability and karyotype changes are of predominant occurrence[6-11]. Cytological and biochemical studies on callus cultures are useful in finding out the role of growth hormones in the production of chromosomal aberrations and in studying nucleic acid metabolism. A decrease in the frequency of dividing cells and increase in polyploid cells were seen with increasing age of culture. The polyploid cells may appear owing to either endomitosis or failure of cytokinesis and subsequent nuclear fusion, or a combination of both. Cell cultures with a mixed population of diploid and polyploid cells have been reported from time to time[12]. Spindle irregularities have been said to account for the occurrence of polyploid and aneuploid cells in callus cultures[13]. In the present study, the polyploid cells were mainly tetraploid and cells with even higher

Table 1 *Mitotic index and frequency of chromosomal abnormalities in Trigonella foenum-graecum cotyledon callus*

	Days in culture				
	31	57	82	105	130
Mitotic index	2.69	2.17	2.07	2.02	1.77
Normal	90.1	89.2	87.1	78.5	68.3
Bridges	8.2	8.9	9.0	15.0	20.3
Laggards	1.7	1.9	3.1	5.3	8.6
Multipolarity	—	—	0.8	1.2	2.8
Mitotic cells scored	3005	2901	2850	2768	2931

ploidy occurred at a much lower frequency. A general tendency of change in ploidy under *in vitro* conditions has also been reported earlier[14].

Different types of ana-telophase anomalies noted in *T. foenum-graecum* were found to increase with duration of culture but the frequency was low. Lagging chromosomes and multipolar separation have been cited as the main source of variation of chromosome number[15]. Such variations appeared partly as the manifestation of nuclear conditions in the primary explant and partly owing to nuclear changes occurring at the time of callus induction[16].

It can be inferred that the cotyledon is the best material for morphogenetic studies in *T. foenum-graecum* under culture conditions. The rapid polyploidization in the callus of *T. foenum-graecum* may be utilized for regeneration of polyploid plants for successful exploitation of its medicinal properties.

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ANNOUNCEMENT

26TH ANNUAL CONVENTION OF THE INDIAN GEOPHYSICAL UNION AND SEMINAR ON GLOBAL CHANGE

The Indian Geophysical Union will hold its 26th Annual Convention during 14–16 December 1989 and organize a seminar on the theme 'Global Change'. Contributed original research papers are invited for presentation in the following general sessions of the Annual Convention: (i) solid earth geophysics/processes, (ii) oceanography, (ii) atmospheric sciences, (iv) space science and planetology, (v) solar–terrestrial relations, (vi) instrumentation.

The seminar on 'Global Change' will have invited papers. However, original research contributions are also welcome.

Abstracts must be sent by 30 October 1989 and full papers by 30 November 1989. For more details contact: Dr D. Atchuta Rao, Hon. Secretary, Indian Geophysical Union, NGRI Campus, Hyderabad 500 007, India.

CYTOGENETIC MONITORING OF OCCUPATIONALLY RADIO-EXPOSED WORKERS IN A RARE-EARTH PLANT

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ABSTRACT

In order to assess risk of cancer among occupationally radio-exposed plant workers, a pilot study was undertaken at the Indian Rare Earth Limited, Chhatrapur. Blood smears from 309 persons (both unexposed and exposed groups) were screened for presence of micronuclei. Dry mill (where thorium is separated from monazite sands) workers showed a higher incidence of micronuclei in their RBC than workers employed elsewhere in the plant. This study indicates that cancer risk persists among the workers owing to exposure to small doses of gamma radiation emitted from the black monazite sands.

INTRODUCTION

THE extent to which human somatic and germinal mutation frequency may be increased by exposure to ionizing radiation and to the variety of chemicals that characterize life in modern societies has been a matter of concern in recent years. Somatic mutations, either genic or chromosomal, are not transmitted to the offspring of an exposed individual. However, increases in these mutations may contribute to an increase in the frequency of acquired disorders, for example cancer. Therefore there is a clear need to develop and apply methods to study exposed populations that are at risk of increased levels of somatic or germinal mutations.

Any effort to determine whether an increase in mutation rate has occurred in a given population must contend with formidable problems, one of the most significant of which is to define the genetic end-points suitable for the study. In the present investigation, on workers in a rare-earth plant, we have chosen the presence of micronuclei in circulating erythrocytes as the genetic end-point of aberrations induced by exposure to ionizing radiation emitted by the monazite sands processed at the plant.

The Indian Rare Earth (IRE) Ltd, Chhatrapur, extracts rare earths from the black monazite sand that is deposited in high concentration in a nearby beach strip covering 1.5 km. The black sand contains ilumenite, garnet, rutile, zircon and monazite. The monazite is enriched with thorium (^{232}Th), a radioactive element that emits gamma radiation. In the dry mill of the plant, thorium is extracted for use as fuel in nuclear reactors.

At present only seven agents (alkylating cytostatic drugs, benzene, epichlorohydrine, ethylene oxide, ionizing radiation, styrene and vinyl chloride) are confirmed as causal for chromosomal damages in humans[1]. However, only five of these agents (excluding epichlorohydrine and vinyl chloride) are known to induce micronuclei in exposed cells. The cells most frequently used are peripheral lymphocytes. Use of RBC in peripheral blood also seems to be promising[2-4]. In the present investigation, blood smears were screened for micronuclei in erythrocytes to determine whether occupationally radio-exposed workers constitute a population group with high risk of cancer.

MATERIALS AND METHODS

The study involved screening 309 persons divided into three groups.

Group I, 110 unexposed persons (age 8 to 60 years). Families of the IRE employees living in the colony at the outskirts of the plant.

Group II, 153 plant workers (age 12 to 60 years).

Group III, 46 dry mill workers (age 22 to 40 years).

Groups II and III comprise the occupationally radio-exposed persons. Although the plant is mechanically operated workers in different sections are likely to come in close contact with rare earths during the final stages of isolation, shifting and packaging. The situation is the same with the dry mill workers also. Thus there are two categories of workers: (i) those employed in different sections of the plant, and (ii) those employed in the dry mill where thorium is extracted. Workers in both groups are exposed to ionizing radiation for 8 h a day.

Personal histories including age, duration of employment and smoking habit were recorded. Each individual was assigned a code number (IREH). For comparison only subjects between 25 and 35 years of age were taken into consideration. The numbers of such subjects (n) in the different categories were 33 (group I), 65 (group II) and 42 (group III).

Blood was obtained by finger-tip puncture and was smeared on a clean greese-free slide. The smear was stained with Leishman's stain[4]. For each individual 1000 RBC were examined to determine the frequency of micronuclei.

RESULTS

The data are given in table 1. Unexposed subjects had only 1–5 RBC with micronuclei out of 1000 RBC examined. The number of RBC with micronuclei varies from 5 to 38 in group II subjects. The highest number (38) was found in a 30-year-old worker (IREH 82) who has been employed for 12 years in the plant. The mean percentage of RBC with micronuclei is six times that for unexposed individuals. Group III subjects had 14–41 RBC with micronuclei per individual (1000 RBC). The highest number was found in a 30-year-old worker (IREH 160) employed in the dry mill for 6 years. The mean percentage of RBC with micronuclei is eight times that for unexposed individuals.

DISCUSSION

A number of recent studies have shown that peripheral lymphocytes of persons exposed to different types of ionizing radiation also have induced chromosomal aberrations. It has been reported that the frequency of chromosomal aberration is more in monazite factory workers involved in handling and processing than in those not handling monazite[5]. The frequency of unstable chromosomal aberrations like dicentrics and rings in nuclear dockyard workers has been reported[6]. There is extensive literature on the use of the lymphocyte assay to measure the dose of different types of radiation[7, 8]. However, there are limiting factors in the use of the lymphocyte assay. For identification of groups potentially exposed (high-risk) to ionizing radiation, the micronucleus assay in peripheral erythrocytes seems to be more convenient, less time-consuming and inexpensive. Exfoliated human cells of the oral cavity have recently been used in a micronucleus assay[9, 10]. Genotoxic effects of tobacco can be detected in buccal mucosa cells of tobacco chewers. Such tests have suggested a causal relationship between oral cancer and the habit of chewing tobacco[9]. Micronuclei have also been reported in peripheral erythrocytes of black rats from high background radioactive areas. The appearance of micronuclei in the RBC has been attributed to the effect of the high background radioactivity of the place on the haemopoietic system of the rats[4].

Table 1 *Mean frequencies of erythrocytes with micronuclei (MN) in blood smears of unexposed and radio-exposed rare-earth plant workers*

Population group	No. of persons screened	Length of employment	Cells with MN (%)	Range of no. of MN/1000 RBC
I (Unexposed)	33	—	0.3±0.012	1–5
II (Plant workers)	65	6 months to 13 years	1.7±0.074	5–38
III (Dry mill workers)	42	2 years to 9 years	2.5±0.054	14–41

Significance of difference, $P < 0.01$.

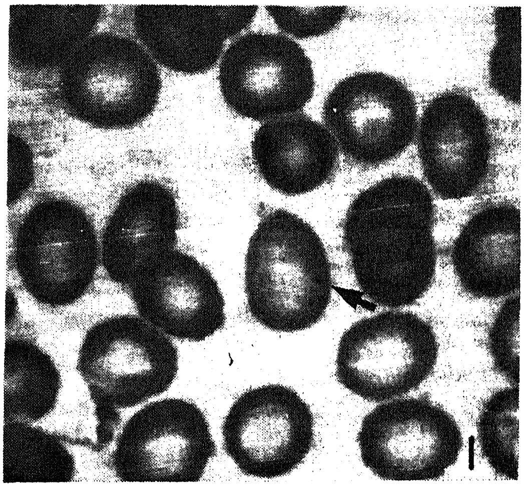

Figure 1. RBC with micronucleus from a radio-exposed individual.

Results of the present investigation show that the micronucleus frequency is about 6 times higher in plant workers and about 8 times higher in dry mill workers than in unexposed individuals. This indicates that dry mill workers constitute a higher-risk group compared to the other plant workers. In the present study we screened 107 persons with 6 months to 13 years of service (table 1). There exists no correlation between the length of employment and the micronucleus frequency. The study indicates that the appearance of micronuclei in RBC is a marker of the hazardous effects of ionizing radiation on precursor bone marrow cells (blast cells) of exposed persons, who constitute an occupationally high-risk group.

ACKNOWLEDGEMENTS

One of the authors (GM) thanks CSIR, New Delhi, for a fellowship. The authors thank Dr P. K. Mohanty, Deputy Chief Medical Officer, IRE Ltd, Chhatrapur, for help during sample collection.

26 July 1988; Revised 15 November 1988

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ANNOUNCEMENT

THIRD CECRI CONFERENCE ON LUMINESCENCE

The Central Electrochemical Research Institute, Karaikudi, will organise the 3rd CECRI Conference on Luminescence on the pattern of the familiar Gordon Research Conferences during January 12–14, 1990 at CECRI, Karaikudi. The conference will provide a forum for discussions in all areas of luminescence phenomena (photo-, cathodo-, thermo-, bio-, electro-, chemi-, triboluminescence), chemistry and physics of phosphor materials, devices such as EL panels, TLDS, and related topics. The conference is limited to 100 participants, including 50 invitees from all over the world. For further details, please contact: Dr R. P. Rao, Convener, 3rd CECRI Conference on Luminescence, Central Electrochemical Research Institute, Karaikudi 623 006, India.

CURRENT SCIENCE—50 YEARS AGO

AGRICULTURE AND ANIMAL HUSBANDRY IN INDIA*

The report of progress in agriculture and animal husbandry in India during the year 1936–37, issued under the authority of the Imperial Council of Agricultural Research, affords as usual a comprehensive summary of agricultural development in all its aspects and forms, like its predecessors, a very useful book of reference to all those interested in the agriculture of this great country. Progress has been so many-sided and scientific activities so numerous that it is no easy task to make a connected and comprehensive narrative of all of them in their due perspective, but the Council has succeeded very well in the attempt. The report, however, relates to work which is now nearly two years old, and though the delay is somewhat unavoidable, one wishes that some arrangement may be made by which future reports can be published without such a long interval elapsing. The information is grouped and dealt with along the same lines as in former reports, with the exception that more attention is devoted to "Fruit development" and "Fodder crops and grazing", which are each given a special chapter.

Considerably more money was made available for agricultural research and development during the year; the total gross expenditure for the whole country rose by Rs. 18 lakhs for agriculture and by Rs. 5 lakhs for veterinary science. The Central Government also made a special grant of the large sum of Rs. 2.82 crores for rural development out of which agriculture has had its due share. The year was notable for the visit of Sir John Russell and Dr N. C. Wright to India to examine and report on the work carried on by the Imperial Council of Agricultural Research. The recommendations contained in their reports which are of a far-reaching character, are summarised in this report, but the action taken or proposed to be taken in respect of these recommendations which are really what would interest one more, do not come within the purview of this report. As regards the adoption of improvements by cultivators, notable progress has been reported in the cultivation of improved varieties of the various crops; the area under such varieties rose from 21.4 million acres in 1935–36 to 23.9 million acres in 1936–37, though this by no means represents the whole extent of such increase. Extensive and well-organised schemes of seed distribution following on crop improvement work by all the departments, central and provincial, continued to make steady progress. Similar progress is reported in respect of fertilisers; the import of chemical fertilisers rose from 72,210 tons to 83,653 tons in the year. Though the consumption of sulphate of ammonia rose from 57,164 tons to 76,360 tons, the share of local production was only 17,748 tons. Local production indeed recorded a slight fall from 17,851 tons to 17,748 tons. One would wish to see the day when India's requirements of phosphatic and nitrogenous fertilisers at least will be fully met from Indian production. In respect of improved implements progress has been moderate, but the redeeming feature is that they are of Indian manufacture, a development which is showing encouraging signs of expansion. Progress in well-boring operations and the installations of pumping sets for irrigation makes interesting reading, both in the variety of operations and in the success that has attended them. In respect of crops aside from the various manurial, cultural and crop improvement investigations, those relating to "quality" deserve special mention and results of considerable practical value are already reported in respect of one, *viz*., rice. This line of work is eminently worthy of expansion so as to embrace other crops as well and more of their "quality" characters. Questions relating to fodder and grazing were the subjects of discussion at three conferences in the year at which forest grazing, improvement of grasslands, better utilisation of waste lands and kindred matters were gone into and a recommendation made for the establishment of a central and provincial fodder and grazing committees for paying special attention to these matters. Under "Fruit development" the results of work on cold storage being conducted in Poona are notable, their practical application in the fruit trade of the country will no doubt soon follow. Progress is reported in many fields of animal husbandry especially in respect of the supply of breeding bulls. A compilation of the

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*Published in *Curr. Sci.*, 1939, vol. 8, p. 234.

type characters of certain important breeds of cattle and buffaloes was arranged for in the year. Many of the numerous investigations under crops, pests and diseases, dairying and cattle improvement were in varying stages of progress and many of them are referred to in broad outline. Forty schemes were completed and there were as many as 136 schemes in the course of investigation at the end of March 1937—a fact which shows that after all agricultural research is not altogether neglected in India. The chapters on agricultural education, veterinary education, District-work and agricultural co-operation give relevant information for the year under report and a number of appendices furnish much statistical details.

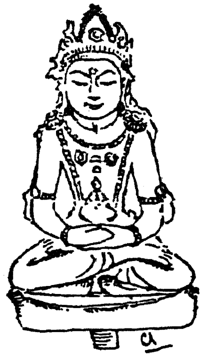
A. K. Y.

A METAL IMAGE OF MANJUSRI*

Manjusri had enjoyed a chief place in the Buddhist Polytheism. The cult of Manjusri seems to have originated in India but, when it was introduced into China, had taken a different form; and it was from China that the cult had spread to Nepal. In Mahayanism, Manjusri is regarded as a Bodhisattva[1].

It is difficult to determine the date of the introduction of this deity into the Buddhist pantheon. However, the Chinese pilgrims, namely, Fa-hien, Hiuen Tsang, and I-tsing, speak of the worship of Manjusri in India.

The *Sadhanamala*[2], a work in Sanskrit, contains *Dhyanas* describing several forms of Manjusri; and the most common representation of this form in sculpture carries the Sword and the Book in its right and left hands.



A metal image of Manjusri, measuring two and a half inches in height, comes from Kanchi (Conjeevaram) having been discovered there (I am informed) in a gutter. The pedestal on which this image must have been installed is now lost and the image itself is faintly corroded at the back. Though small, the figure looks beautiful and was probably (judging by its size) under private worship and discarded later by someone.

That Kanchi had been a great centre of Buddhist faith and that many schools of Buddhist philosophy flourished therein are only too well known. A large number of stone images of the Lord have been noticed here but a figure of Manjusri representing a particular type of Mahayanism has not been so far traced. This metal image, therefore, appears to be a valuable archaeological find.

One form of Manjusri has the nomenclature of Vak or Vajraraga which is described by Bhattacharya[3] as follows:

> Vajraraga or Amitabha Manjusri is one-faced and two-armed. His hands are joined in the lap, forming the *Dhyana* or *Samadhi Mudra*. In this respect he is identical with his Sire whom he bears on his tongue. But he may be distinguished by the ornaments he wears and by the image of his Sire if represented on the crown. . . . . . Images of this form of Manjusri are extremely rare in India with the exception of the one at the temple of Bauddhanath in Nepal.

The special features which characterise this image are (1) *Mudra-Samadhi*, (2) *Asana-Vajraparyanka*, (3) Ornaments and Dress.

The image from Kanchi seems to agree well with the description given above of Vak or Vajraraga Manjusri with the exception of an object which the figure holds in its hands. Even in the case of the Manjusri whose figure is reproduced by Bhattacharya, a more or less similar kind of object is visible though

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*Published in *Curr. Sci.*, 1939, vol. 8, p. 218.

unexplained by the author. As regards the Conjeevaram image, the object appears to be a human head which, perhaps, represents the head of Amitabha or the Sire on whom the Manjusri is supposed to concentrate.

Since the provenance of this image is Kanchi we may suppose that Manjusri cult was prevalent in this historic city in the early centuries of the Christian era. The very fact that Kanchi attracted the attention of Vajrabodhi, a Great Worshipper of Manjusri, proves the existence of Tantric form of Buddhism in Kanchi in the seventh century A.D. In this connection it is well to remember the statement of the late Gopinatha Rao who says that the famous Kamakshi temple was originally dedicated to the Tantric Goddess Tara[4].

C. MINAKSHI

Madras
May 2, 1939

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SHORT COMMUNICATIONS

WEAK GAMMA RAYS OF ^{75}As

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$^{75}_{34}$Se (127 days) decays by electron capture to several excited states of $^{75}_{33}$As. The gamma transitions of various energies ranging from 15 to 821 keV have been reported earlier[1-3]. However, controversies about the low-energy, weak gammas for 15 keV and 81 keV have appeared in the literature[2]. The decay scheme has been studied using NaI(Tl) counters and Ge(Li) detectors. The γ–γ coincidence and angular correlation techniques have also been attempted[1-3].

In spite of the controversies over the excited levels of ^{75}As, the authors disagree over the values of the intensities of the low-energy gammas[2]. It is thus interesting to investigate the controversial gamma transitions in the spectrum of ^{75}Se. An efficient HP–Ge detector has been used for the study of the low-energy gamma transitions.

Figure 1 shows the gamma spectra of ^{75}Se measured with the HP–Ge detector. The resolution for the 133 keV γ-ray photopeak was estimated to be 2%. The pulse height spectrum ranging from 15 to 81 keV is shown in figure 1. The controversial transitions of 15 keV and 81 keV do appear in the spectrum. A least-square fit was applied for the calibration of the spectrometer and necessary corrections for the efficiency of the detector were made to calculate the relative intensities of gammas. The relative intensities of gammas were estimated with respect to the intensity of the 66 keV gamma, which was normalized to 1 (table 1). Table 1 also gives the relative intensities found by others[2], with the intensity $I_\gamma(66)$ normalized to 1 for comparison.

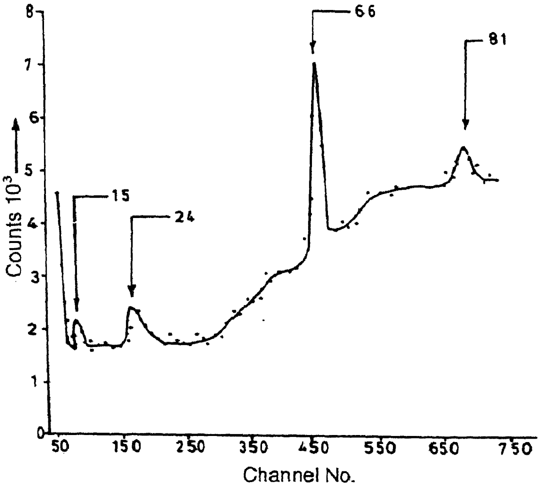

Figure 1. Gamma ray spectrum of ^{75}Se measured with HP–Ge detector (gamma energies are in keV).

Table 1 *Gamma ray intensities relative to $I_\gamma(66) = 1$ in spectrum of ^{75}Se*

E_γ (keV)	Present work	Ref. 2
15	0.04 ± 0.020	0.007 ± 0.002
66	1.00	1.00
81	0.1 ± 0.033	0.022 ± 0.004

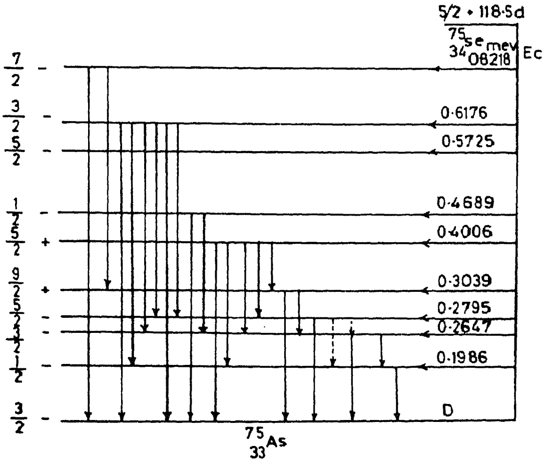

Figure 2. Decay scheme of $^{75}_{34}$Se. (Dotted lines show the disputed gamma transitions).

The HP–Ge detector yields better results compared to others[2]. Figure 2 shows the decay scheme of ^{75}Se with the disputed gamma transitions[4].

The authors thank Prof. B. B. Balliga, Saha Institute of Nuclear Physics, Calcutta, for providing Ge-detector gamma spectra.

9 September 1988; Revised 30 December 1988

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TWO UNDESCRIBED FUNGI ON ORCHIDS

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DURING a study of fungal diseases of ornamental plants, an unusual leaf spot disease was noted on two beautiful orchids, *Cymbidium* sp. and *Dendrobium* sp., incited by two coelomycetous fungi, species of *Haplosporella* Speg. and *Phomopsis* Sacc. A detailed study revealed that these two fungi are undescribed from the above two hosts or others in the family Orchidaceae so far[1–4]. Hence, these are being described here as new taxa on the basis of comparative morphology and host relationship[5].

Haplosporella cymbidii sp. nov. (figure 1)

Area necroticae irregulares, in strias longus depositae, laevis margo, hypophyllae, bulbalinus in medio et marginibus, stromurimus, coalitus. Pycnidia dispersis, punctiformae, fuscabrunnoea vel nigris, sub-erumpentia, 191–245.5 μm diam., paraphysoides inter-mixtus; pycnidiosporae brunneae, incrassatus, elliptica vel obovata, laevia, magnit 18–23 × 10–13 μm.

Pycnidia scattered, hypophyllous, punctiform, dark brown to black, sub-erumpent, wall composed of few layers, inner wall lined with narrow elongated sterile threads, measure 191–245.5 μm; pycnidiospores pale brown, thick-walled, oval to obovate with truncate base, measure 18–23 × 10–13 μm.

On living leaves of *Cymbidium* sp. Leg. B.R.D.Y., June 1977, at Pune, AMH-4135 (holotype).

Phomopsis dendrobii sp. nov. (figure 2)

Area necroticae, sub-circulares vel angulosae, dispersae, in centro expallescentes, 2–3 perfolium,

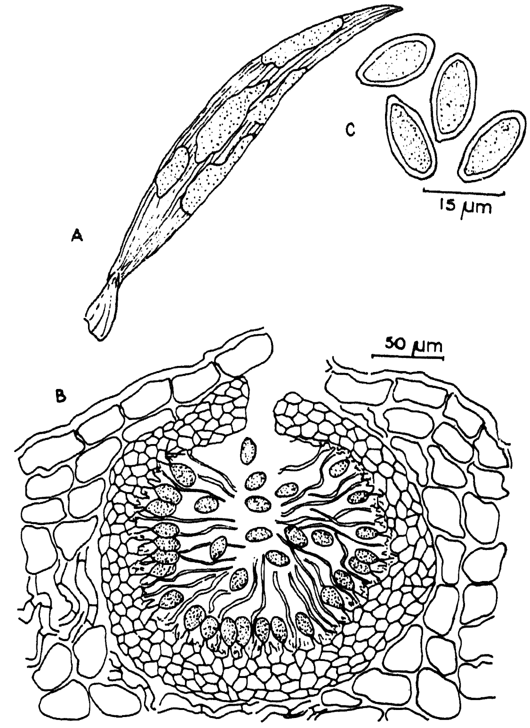

Figure 1. *Haplosporella cymbidii.* **A,** Habit; **B,** VS of pycnidium; **C,** pycnidiospores.

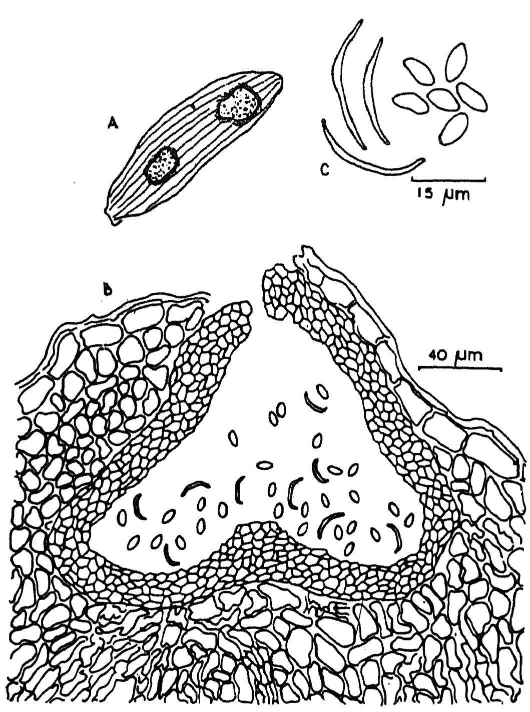

Figure 2. *Phomopsis dendrobii.* **A,** Habit; **B,** VS of pycnidium; **C,** the two types of pycnidiospores.

laevis margo, non-coalitus, salmoneus in medio et castaneus ad marginem fusco-brunnea. Pycnidia punctiformae, sub-globosa vel ampuliformae et irregularlis, immersa, ostiolata, magnit 130–290 μm diam; pycnidiosporae, bi-typus: (a) sporae simplicia, hyalina unicellularia, laevia, elliptica, 6.7–9.5 × 4 μm; (b) sporae hyalina, unicellularia, laevia, fulcata vel allantoidea, apicibus, subulate, 26.5–39.5 × 3.3 μm.

Pycnidia subglobose to irregular, ampulliform, immersed, later becoming erumpent; ostiole opening widely measure 130–290 μm diam., pycnidiospores two types: (a) spores simple, one-celled, hyaline, smooth, elliptical 6.7–9.5 × 4 μm; (b) spores hyaline, one-celled, falcate to allantoid with subulate ends, measure 26.5–39.5 × 3.3 μm.

On living leaves of *Dendrobium* sp. Leg. B.R.D.Y., July 1978, at Pune, AMH 4136 (holotype).

One of the authors (BRDY) is grateful to the Ministry of Education, Government of India, for financial support.

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CULTIVATION TRIALS OF *PLEUROTUS OPUNTIAE* (DUR. AND LEV.) SACC.

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SPECIES of *Pleurotus* are well-known edible mushrooms in different parts of the world. Unlike *Agaricus bisporus*, which is generally used fresh or canned, *Pleurotus* can be dried and stored for long periods without deterioration in culinary properties. In European countries and Japan *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer[1] and in Taiwan *P. cystidiosus* Miller[2] have been cultivated for a long time. Cultivation of various other species has also been reported in the literature[3–6]. The present paper reports cultivation of a wild species which was found growing on dead, decaying stumps of *Opuntia* sp. during the rainy season in 1984 in forests of Garhwal (Himalayas). This mushroom was obtained in pure culture by transferring tissue from the pileus on to yeastal PDA slants. The mycelium grew well between 25 and 28°C. Growth of the colony was fast and vigorous, and aerial growth of hyphae was also seen. The spawn was made by the usual method on wheat grains. The cultivation was carried out on rice straw substrate in polybags of size 75 × 45 cm holding 4 kg wetted straw (one kg dry). One hundred grams of grain spawn was mixed with the substrate prior to filling in the bags, which were perforated all over for ventilation. The mouths of the prepared bags were tied and the bags were placed on a shelf in a mushroom house maintained at 25–28°C. The substrate was completely colonized by the mycelium in 10–12 days. At this stage the polythene was torn off and removed completely, leaving a compact mass of mycelium-covered straw. Watering was done twice a day by gentle spray. Humidity of the growing room was kept at 80–90% and temperature at 20–25°C.

After 15–20 days of spawning fruit bodies started to appear and matured in another 2–3 days. Mushrooms continuously appeared at intervals; total yield after the cropping period of 25–30 days was 750 g net (in 1 kg dry rice straw). These mushrooms were graded as fair to good in culinary properties by qualified persons. Compared to *P. sajor-caju*, these mushrooms are more fleshy and tasty. Their snow white colour is an added advantage as this increases commercial acceptability. The description of the mushroom is given below.

Basidiocarp pleurotoid, imbricate, single or in clusters (figure 1). Pileus fleshy, 6–12 cm in diameter, depressed at the point of attachment to the stalk, colour white, paler towards margin, surface tomentose or glabrous, margin irregular, lobed and upcurved at maturity. Stipe eccentric, rarely central, 3–5.3 cm long and 4–9 mm across, cylindric, white, tomentose. Gills decurrent, white to cream-coloured, crowded, 2 mm broad, partial veil absent,

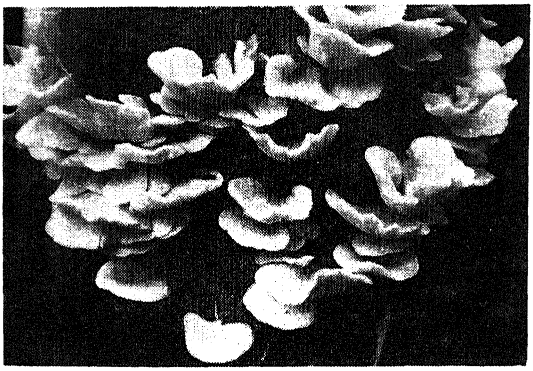

Figure 1. *Pleurotus opuntiae.*

context fleshy. Hymenium with thick-walled, irregularly arranged hyphae, inamyloid, clamp connections present, sub-hymenium with thin-walled hyphae. Spore print white creamy. Spores hyaline, cylindric, ellipsoid, non-amyloid, thin-walled, 8–11 × 3.5–4.7 μm, cystidia absent.

The above description completely matches with the description given by Pegler[7]. The fungus was identified as *P. opuntiae* (Dur. & Lev.) Sacc. and the culture was deposited in the Indian Type Culture Collection, Division of Mycology and Plant Pathology, IARI, New Delhi, under Accession No. ITCC 3311.

This note forms part of the Ph.D. thesis submitted by (DB) to the University of Garhwal.

19 December 1988

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PHYLLODY OF *TEPHROSIA COCCINEA* WALL. (FABACEAE)

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TEPHROSIA COCCINEA is an undershrub distributed in western peninsular India[1,2]. It is rather rare in Marathwada region on the Deccan Plateau. Normal plants have 1–5-foliolate leaves with oblanceolate, emarginate leaflets that are glabrous and dark green on the adaxial surface and have densely appressed grey hairs abaxially. The flowers are pink and borne in leaf-opposed or terminal short racemes (figure 1a).

A peculiar individual of this species was collected in the Marathwada University Campus. In this plant the leaves were quite normal but all the flowers were replaced by clusters of bract-like outgrowths (figure 1b). All the whorls of floral appendages were transformed into green, ciliate bracts (figure 1c,d).

A review of the literature on plant diseases and teratomorphosis reveals that such abnormal and sterile specimens have been reported in a large number of cultivated as well as wild plants. The present specimen of *Tephrosia coccinea* is thus an addition to the list of abnormal specimens. A series of abnormalities progressing from a normal flower to completely vegetative branch has been termed antholysis[3]. This includes stages such as virescence (greening of floral parts), phyllody (development of floral parts into normal foliage), apostasis (the development of internodes theoretically present in the floral receptacles) and prolification (elongation of receptacle above the insertion of the pistil).

In the present case all the floral parts have been transformed into bract-like appendages. Abnormalities very similar to these have been particularly reported in *Crotalaria juncea* (Fabaceae)[4], *Sesamum indicum* (Pedaliaceae)[5], *Emilia sonchifolia* (Asteraceae)[6] and a few other plants. But the abnormal stages have been variously termed as phyllody[4,5] or invirescence[6].

At present nothing definite can be said about the abnormality in *T. coccinea* but it would be appropriate, on the basis of symptomology, to regard the present phenomenon as that of phyllody. In the various plant species mentioned above, the abnormal development of flowers has been reported to be due to infection by a virus or a mycoplasma-like organism. There is, however, a greater tendency to ascribe such plant abnormalities to the latter[7], especially after 1967.

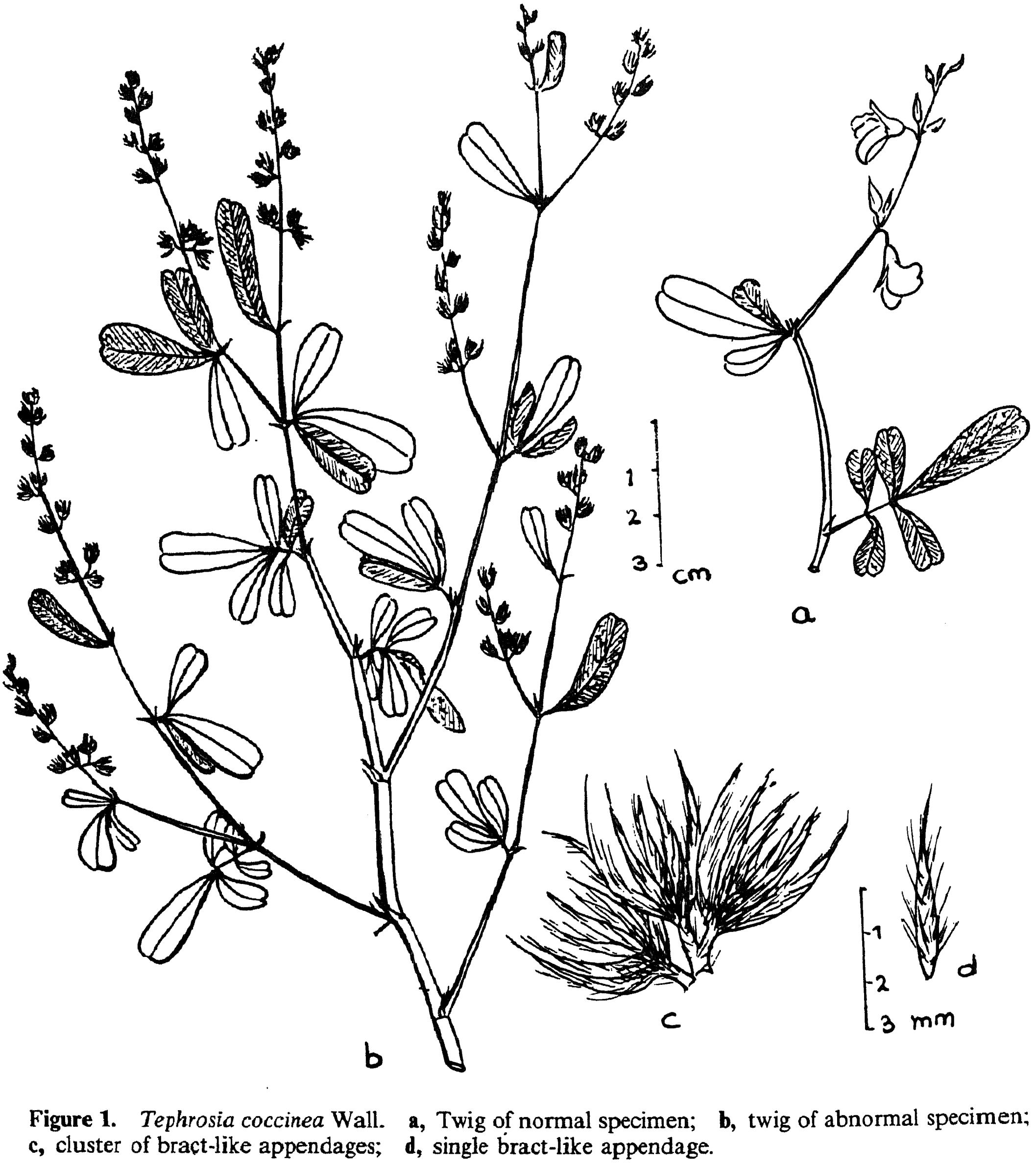

Figure 1. *Tephrosia coccinea* Wall. **a,** Twig of normal specimen; **b,** twig of abnormal specimen; **c,** cluster of bract-like appendages; **d,** single bract-like appendage.

Incidentally, it may be noted that the causal organism of the abnormal development of flowers appears to be very specific as the abnormality has not been observed in any other plant in the nearby vicinity, not even in allied species such as *T. hamiltonii* Drum. or *T. villosa* (L.) Pers., which are very common in the area. As far as the present author is aware, such an abnormality is being reported for the first time in *T. coccinea*.

24 October 1988

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LOCALIZATION OF UREASE IN *ANABAENA DOLIOLUM*

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THE degradation of urea by micro-organisms is effected by either urease (urea amidolyase, EC 3.5.1.5) or ATP-urea amidolyase (UALase)[1]. The latter enzymatic process, also found in yeast and green algae, involves two distinct enzymatic activities: urea carboxylase (urea: CO_2 ligase (adenosine 5-diphosphate forming), EC 6.3.4.6) and allophanate hydrolase, EC 3.5.1.13. All blue-green algal species studied so far contain urease[2-4]. Urease from a bacterial system is a soluble enzyme located in the cytoplasm[5]. The present paper describes the localization of the enzyme urease in the blue-green alga, *Anabaena doliolum*.

A. doliolum was grown in modified Chu No. 10 medium[6] supplemented with 1 mM urea. The cultures were incubated at 24 ± 1°C and illuminated with daylight fluorescent tubes (intensity 2500 lux on the surface of the vessels) for 14 h per day. For preparation of cell-free extract, exponentially growing algal cultures collected by centrifugation were washed repeatedly with sterile distilled water and finally with 5 mM phosphate buffer, pH 7.3. The cells were ground in a pre-chilled glass mortar and pestle at 4°C in an ice bath with an equal volume of acid-washed sand. Crude enzyme was extracted with 5 mM phosphate buffer, pH 7.3. The supernatant collected after centrifugation was used for estimation of urease activity. For preparation of spheroplasts, algal cells were treated with ethylenediaminetetra-acetic acid–lysozyme according to Neu and Heppel[7], with the modification that a 20% glycerol solution was substituted for sucrose solution to protect the spheroplasts[5]. Urease was estimated by determining the amount of urea decomposed within a given period of time at 37°C (pH 7.3). The reaction mixture contained 2 μmoles of urea in a final volume of 2 ml of 5 mM phosphate buffer (pH 7.3). The reaction was initiated by the addition of cell-free enzyme extract and terminated by the addition of 4 ml of mixed reagent used for the colorimetric assay of urea[8]. Protein was determined by the method of Lowry *et al.*[9] using bovine serum albumin as the standard.

Table 1 *Urease activity in whole cell extract and spheroplasts of Anabaena doliolum*

Fraction	Urease activity (nmol urea hydrolysed per mg protein per h)	Activity as percentage of that in cell extract
Whole cell extract	652.0	100.00
Spheroplast lysate	591.0	90.64
Supernatant (glycerol–tris buffer after sedimentation of spheroplasts)	15.0	2.30

Table 1 gives urease activity in whole cell crude extract, spheroplast lysate, and supernatant obtained after sedimentation of spheroplasts. Since most (90%) of the urease activity was found in spheroplast lysate (released from spheroplasts after osmotic shock and lysis), urease of *A. doliolum* is present in spheroplasts as a soluble enzyme.

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SENSITIZATION OF *ASPERGILLUS PARASITICUS* SPORES TO GAMMA RADIATION IN PRESENCE OF HYDROGEN PEROXIDE

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AFLATOXINS, produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*, are considered to be the most carcinogenic natural substances. Contamination of different crops by these fungi and aflatoxins is a serious problem all over the world, and no method for an effective aflatoxin control is known to date[1]. Various methods for detoxification of aflatoxin have been described[2]. The effect of gamma radiation on spores of *A. parasiticus* and aflatoxin production has been studied earlier[3,4]. Recently we have reported an effective detoxification system for aflatoxin by the synergistic effect of hydrogen peroxide and gamma radiation[5]. In this communication we report sensitization of *A. parasiticus* spores to gamma radiation in presence of hydrogen peroxide.

A. parasiticus NRRL 3240 was grown on agar containing glucose, 4%, and peptone, 1% (pH 4.5). Spores were harvested after 7 days of growth and the spore suspension was adjusted to 10^7 spores/ml in saline. Irradiation was carried out in a gamma chamber 900 (provided by Bhabha Atomic Research Centre, Bombay, India) at 28 ± 2°C and a dose rate of 6.6 krad/min. The irradiation source was ^{60}Co. Spore suspension, in saline or in 0.1, 1 or 5% hydrogen peroxide, was irradiated at different doses. Small portions of suspension were removed and the surviving population was determined using the standard plate count method. Spores were also incubated in 0.1, 1 and 5% hydrogen peroxide for different times and their viability checked as above. The surviving population was grown on agar plates and screened for mutants unable to produce aflatoxin as described by Aybe *et al.*[6]

The survival curve for spores of *A. parasiticus* irradiated in saline (figure 1) gives a D_{10} value (90% killing) of 38.75 krad. Similar results were also obtained by Sharma *et al.*[3] In our detoxification system[5] we had used 5% hydrogen peroxide and gamma radiation. When irradiation was carried out in presence of 5% hydrogen peroxide, at 50 krad the surviving population was found to be less than 10% (data not presented). Hydrogen peroxide is a widely used surface-sterilizer and disinfectant. Its killing effect on spore-forming and non-spore forming bacteria is well documented[7].

It was shown that 3% hydrogen peroxide could bring down the population to 0.001% in 15 min. The present results (table 1) suggest that hydrogen peroxide is effective against spores of *A. parasiticus*. The surviving fraction of spores after a 10-min incubation in the presence of 5% hydrogen peroxide

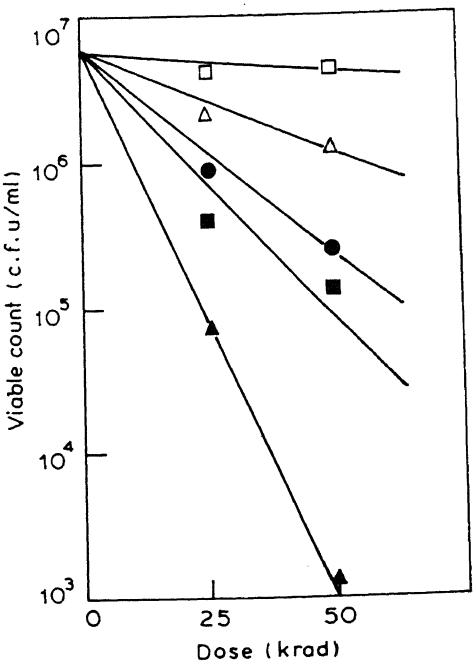

Figure 1. Survival curves for *A. parasiticus* spores irradiated (●——●) in saline, (■——■) 0.1% hydrogen peroxide, (▲——▲) 1% hydrogen peroxide, or incubated without irradiation (□——□) in 0.1% hydrogen peroxide and (△——△) 1% hydrogen peroxide.

Table 1 *Effect of hydrogen peroxide on viability of spores of A. parasiticus*

H_2O_2 (%)*	Viable count	Per cent killing
0	2.5×10^6	0
0.1	1.5×10^6	40
1.0	1×10^6	60
5	1×10^5	96

*Spores were incubated in saline or hydrogen peroxide for 10 min. Viable counts shown are mean of triplicates.

was 4%; in presence of 1 and 0.1% hydrogen peroxide it was 40 and 60% respectively. Spores were also irradiated in presence of 1 and 0.1% hydrogen peroxide and the survival curves were obtained (figure 1). The D_{10} values are 10.6 krad and 30 krad for 1 and 0.1% hydrogen peroxide respectively. Surviving spores were grown on agar and the colonies were observed under UV light for the presence of aflatoxinless mutants. Several colonies were checked but none of them showed the aflatoxinless phenotype. Similar results were also obtained by Moreno *et al.*[8] who nonetheless obtained a few mutants that showed partial reduction or altered aflatoxin production.

The results obtained suggest that hydrogen peroxide alone can act as an effective killing agent in the case of *A. parasiticus* spores. The spores also showed very low resistance to gamma radiation and a low D_{10} value in saline. Hydrogen peroxide is one of the radiolytic products during radiation and was shown to function as a spore radiation sensitizer in the case of bacteria[9]. In the present experiments irradiation and hydrogen peroxide showed additive effects (figure 1). This suggests that hydrogen peroxide might be one of the intermediates during irradiation, and addition of hydrogen peroxide enhances sensitization of the spores to gamma radiation. Earlier observations[6], showed that hydrogen peroxide along with gamma radiation is an effective system for detoxification of aflatoxin in contaminated food. Thus, the use of hydrogen peroxide and gamma radiation for treating contaminated foodstuffs would be economical and effective, as it results in killing of *A. parasiticus* spores and in degradation of aflatoxin.

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EFFECT OF DIFFERENT MEDIA ON THE MORPHOLOGY AND CULTURAL CHARACTERISTICS OF *CANDIDA ALBICANS*

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THE morphology of various species of fungi is significantly affected by different physical and nutrient media[1-7]. In the present study morphology and cultural characters of *Candida albicans* were studied in five nutrient media. The aim of the investigation was to identify those morphological characters that are stable and can be used conveniently for the identification and differentiation of species of *Candida*.

Two male individuals, aged 42 and 20 years, were brought to the laboratory complaining of severe itching on the toes. The interdigital portions were erethematous, and in severe cases the skin had peeled off leaving pink spots. Scrapings were taken from the area and cultured, and pure cultures prepared. The KOH test gave positive results. The isolate was identified on the basis of the criteria suggested by Frey *et al.*[8] The test isolate was grown on five different agar media, viz. malt agar, potato dextrose agar, glucose yeast extract agar, rice agar and Asthana and Hawkers agar, and incubated at

Table 1 *Effect of different media on growth and morphology of Candida albicans*

	Malt agar	Potato dextrose agar	Glucose yeast extract agar	Rice agar	Asthana and Hawkers agar
Macroscopic characters					
Diam. of colony (cm)	2.8 × 1.5	2.7 × 1.0	2.1 × 1.8	2.2 × 1.9	2.5 × 2.0
Colour	Cream	White to cream	White to cream	White to creamy	Cream
Shape	Oval	Linear	Oval	Egg-shaped	Oval
Microscopic characters					
Shape of blastospores	Hyaline, 1-celled, mostly ovoid, few ellipsoid	Hyaline, 1-celled, mostly ovoid, few ellipsoid	Hyaline, 1-celled, highly guttulated, mostly ovoid, some ellipsoid	Hyaline, 1-celled, highly guttulated, mostly ovoid, few ellipsoid	Hyaline, 1-celled, ovoid to ellipsoid
Size of blastospores (μm)	3.6–4.0 × 7.3–14.4	3.9–4.2 × 8.0–10.5	3.5–4.1 × 8.0–10.9	4.1 × 8.0	4.2 × 5.0–10.1
Mycelium	Few, septate, branched	Profuse	Abundant, septate, hyaline, branched	Abundant, septate, hyaline, branched	Abundant, septate, hyaline, branched
Pseudomycelium	Branched, guttulated	Branched, guttulated	Profusely branched, guttulated	Rarely produced, hyaline, septate	Rarely formed
Chlamydospores	Abundantly produced	Few produced	Abundantly produced	Produced in clusters, abundant	Rarely produced

28 ± 2°C. The media were prepared according to the formulae given by Ainsworth[9]. The colour, shape and diameter of colonies, shape and size of pycnidia and pycnidiospores, nature of mycelium and pseudomycelium, and quantity of chlamydospores produced were recorded after a week.

It was observed that morphology and cultural characters of *C. albicans* were significantly affected by different nutrient media (table 1). The present findings agree with those obtained earlier[1-7]. Growth rate was variable, but shape and colour of the colonies were more or less stable. Formation of chlamydospores was seen in nearly all the test media. The present findings agree well with the results obtained by Rajak and Rai[6]. Kafi and Tarr[4] reported that conidia of *Helminthosporium* species showed remarkable variation in size on different media. Rajak and Rai[6] found that morphology of pycnidiospores of 18 species of *Phoma* was very different on different nutrient media. Singh[5] reported that size and morphology of the conidia of *Phyllosticta cestri* and *Phyllostictina artocarpina* were similarly affected. In the present investigation also, size of blastospores of *C. albicans* was different on different nutrient media. Dorenbosch[10] used chlamydospores as the diagnostic character for differentiating the species of *Phoma.*

On the basis of the above findings, it is obvious ~~that one~~ character cannot be used for identification of the species of *Candida*; several characters, such as colour and shape of colonies, shape and size of blastospores, characteristics of mycelium and pseudomycelium, and formation of chlamydospores, should be considered.

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IMPACT OF DIFFERENT LIQUID MEDIA ON AMYLASE PRODUCTION BY SEED MOULDS OF PEARL MILLET (*PENNISETUM AMERICANUM* (L.) LEEKE)

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DETERIORATION of seeds is usually attributed to the action of amylase secreted by seed-borne fungi. A study of pearl millet (*Pennisetum americanum* (L.) Leeke) seeds revealed constant association of certain fungi responsible for seed deterioration. The present work was carried out to assess the ability of amylase secretion of these fungi.

Seventeen test moulds were isolated from pearl millet seeds collected from field and storage. Amylase production by the moulds was studied by using three different substrate and non-substrate liquid media. Among the fungi isolated, *Aspergillus flavus* and *Curvularia lunata* were found to be highly efficient in amylase production while fungi like *Cladosporium cladosporoides* and *Memnoniella* sp. were found to be not as efficient. Starch medium was superior, compared to pearl millet flour and glucose media, for amylase production by most of the tested fungi.

The agar plate method was employed for isolation of seed-borne fungi. The substrate was 1% starch in a medium containing 0.25% KNO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$. Flasks containing medium (25 ml) were inoculated with spore suspensions of the test fungi and incubated at 25 ± 1°C for 8 days. The culture filtrates were then collected. Sterilized starch assay agar medium (soluble starch 10 g, Na_2HPO_4 2.84 g, NaCl 0.35 g, agar 15.9 g, distilled water 1000 ml, pH 6.0) was poured into sterile petri plates, and 0.2 ml of culture filtrates were placed in a

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*For correspondence.

Table 1 *Amylase production by seed moulds of pearl millet in three liquid media*

	Activity zone (cm)		
Species	Glucose medium	Starch medium	Pearl millet flour medium
Alternaria alternata	1.4	2.1	1.8
Al. tenuis	1.6	2.5	2.4
Aspergillus flavus	1.7	3.5	1.9
As. niger	2.2	1.6	0.9
Cladosporium cladosporoides	0.0	0.0	0.0
Curvularia lunata	1.5	3.0	2.0
Cu. pallescens	1.4	1.6	1.7
Drechslera longistrata	1.6	1.6	1.9
D. tetramera	2.4	1.9	1.6
Fusarium moniliforme	1.4	2.3	2.0
F. oxysporum	2.0	1.9	1.5
F. sciitectum	1.7	2.1	2.1
Penicillium funiculosum	1.3	2.0	2.0
Pythium sp.	2.5	2.7	2.1
Memnoniella sp.	0.0	0.0	0.0
Rhizoctonia solani	1.9	2.5	2.4
Rhizopus stolonifer	1.9	2.1	1.0

cavity (0.08 cm diameter) in the centre of the plates. After incubation at 28°C for 48 h, the plates were flooded with Lugol's iodine solution. The width of the non-blued zone around the cavity was measured and the results were recorded as the average of two replicates.

It is clear from table 1 that of the seventeen fungi tested, *A. flavus* and *C. lunata* were highly efficient in amylase production, forming 3.5-cm and 3.0-cm zones respectively. *Cladosporium cladosporoides* and *Memnoniella* sp. were not capable of amylase production.

It is interesting to note that the starch medium was superior, compared to glucose and pearl millet flour media, for amylase secretion by all the fungi except *Aspergillus niger*, *Curvularia pallescens*, *Drechslera longistrata*, *D. tetramera* and *Fusarium oxysporum*. Pearl millet flour medium was the test for *C. pallescens* and *D. longistrata*, while glucose medium was the best for *Aspergillus niger*, *D. tetramera* and *F. oxysporum*. In addition to starch, pearl millet contains proteins and lipids. The superiority of pearl millet flour medium over glucose medium can be attributed to the additional nutrients, which probably stimulated amylase secretion. Similar work was done on jowar flour medium by Wadje and Deshpande[7].

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PHYSIOLOGICAL BASIS FOR THE INHIBITION OF NITROGENASE ACTIVITY IN WATER-STRESSED COWPEA (*VIGNA UNGUICULATA* (L.) WALP)

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NITROGENASE activity was found to be extremely sensitive to water stress in many leguminous crops[1-3]. Even a mild water stress (0.5 MPa decrease in plant water potential) leads to more than 50% drop in total nitrogenase activity (TNA) in many crops[1,3,4]. However, other physiological processes like photosynthesis, transpiration and nitrate reduction are comparatively less affected at a similar plant water status[2,4]. But there has been a controversy whether the high sensitivity of nitrogenase activity is due to dehydration effects on the enzyme in the nodules or indirect causes like limitation of photosynthate supply from the shoot. In earlier studies, Huang *et al.*[5] indicated that in water-stressed soybean, photosynthate supply from the shoot essentially limited nitrogenase activity in the nodules. However, more recent studies[6-8] have indicated direct effects of dehydration on the nodules to be primarily responsible for the drop in nitrogen fixation rate in soybean and other annual legumes. In the present communication we report the results of stem girdling experiments designed to evaluate the role of host photosynthate limitation on the inhibition of nitrogenase activity in normal and water-stressed cowpea plants.

Cowpea plants (cv. C-152) were grown in glazed pots of 15 kg capacity filled with loamy sand soil (pH 6.5, organic carbon 0.52% and total nitrogen 0.05%). The pots were kept under natural illumination and temperature in a greenhouse (1500 μmol s^{-1} m^{-2} light intensity, 30–35°C day temperatures and 14-h photoperiod) and irrigated with tap-water. The treatments were imposed when the plants were 30 days old. The pots were divided into two equal sets, and one set was subjected to water stress by withholding water three days prior to sampling, while the other set continued to receive daily watering. Each of these sets was further subdivided into two groups. Plants were girdled according to the procedure described earlier[5] in one group while the other set was left ungirdled. All observations were made 24 h after girdling.

The rates of photosynthesis and transpiration were measured in the uppermost fully expanded leaves of plants in each treatment using a LI-6000 portable photosynthesis system (Licor Inc., USA). Leaf water potential (Ψ_{leaf}) was measured using a Scholander pressure bomb (PMS Instruments Inc., USA). Nitrogenase activity was assayed by acetylene reduction assay (ARA) as described elsewhere[9]. Total soluble sugars in nodules and roots were estimated by the phenol–sulphuric acid method[10] using 200 and 500 mg fresh material respectively. Total ureide content of dried stem and nodules was determined by the phenylhydrazine method following extraction with equal volumes of ethanol and 0.1 M phosphate buffer (pH 7.2)[9].

Both water stress and girdling caused significant reduction in total (TNA) and specific (SNA) nitrogenase activity (table 1). Water stress led to a significant reduction in both fresh and dry weights of nodules per plant, while stem girdling alone resulted in only a marginal decrease in nodule fresh weight. In stressed plants, therefore, part of the reduction in TNA was due to the reduction in fresh weight of the nodules because of shedding, whereas the reduction in SNA in all the treatments is essentially due to the stress effects on the activity in the existing nodules. The significant reduction in SNA of well-watered plants within 24 h after girdling indicates that any short-term impediment in phloem translocation causes severe inhibition of nitrogenase activity in nodules. In this context it may be relevant to mention that short-term limitation of current photosynthate supply was found to restrict nitrogenase activity in soybean[5], while in perennial species like alfalfa reserve carbohydrates in the nodules supported the nitrogenase activity despite complete removal of canopy[11].

Plant water status (Ψ_{leaf}), and photosynthesis and transpiration rates did not differ significantly in stem girdling without water stress, but under water stress there was a significant decrease in all these parameters. However, soluble sugar content in roots and nodules declined significantly due to girdling (table 2), indicating a possible restriction of photosynthate flow from shoot.

In the stressed plants, no difference was found between girdled and ungirdled plants in SNA of nodules. Similarly, rates of photosynthesis and transpiration, and leaf water potential did not differ. However, stressed and girdled plants had lower total soluble sugars in roots and nodules than stressed, ungirdled ones. But stressed plants, girdled or ungirdled, had higher soluble sugars in nodules than well-watered plants. The increase in soluble sugars in water-stressed plants may be due to the hydrolysis of polysaccharides normally associated with water stress or transport of a greater proportion of photosynthate to roots and nodules at the expense of shoot, as was found in soybean[12].

Thus the data show that the direct effects of dehydration on nodules, either causing shedding or affecting the functioning of existing nodules, become overwhelming under water stress compared to all the other factors, including the supply of assimilates

Table 1 *Effect of water stress and stem girdling on photosynthesis, transpiration, nitrogenase activity and nodule biomass of cowpea plants.*

	Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)	TNA (μmoles C_2H_4/plant/h)	SNA (μmoles C_2H_4/g nodule/h)	Nodule dry weight (mg/plant)
No stress, no girdling	0.549	263	2.400	28.58	82.00
No stress, girdling	0.480	251	0.231	4.65	58.67
Stress, no girdling	0.210	167	0.025	1.09	32.00
Stress, girdling	0.260	173	0.022	0.89	30.73
± SE of mean	0.082	14	0.066	0.67	5.82

Table 2 *Total soluble sugar and ureide content of different plant parts of cowpea under water stress and girdling*

	Total soluble sugars ($mg\ g^{-1}$ dry wt)		Total ureides ($\mu mol\ g^{-1}$ dry wt)	
	Nodules	Roots	Nodules	Shoots
No stress, no girdling	68.20	36.23	5.41	41.42
No stress, girdling	45.83	31.13	6.91	18.08
Stress, no girdling	104.60	69.40	13.61	67.71
Stress, girdling	66.50	48.40	12.74	61.25
±SE of mean	6.07	6.46	1.21	2.65

from the shoot. The drastic reduction in TNA under stress therefore may not be primarily related to the availability of carbon skeletons *per se* in the nodules. The reduction in nitrogenase activity may have to do with enzymes concerned with the supply of energy and carbon skeletons to the nitrogenase reaction in nodules besides effects on nitrogenase itself. Limitation of oxygen supply to the bacteroids due to structural changes in stressed nodules was shown to be an important factor affecting nitrogenase activity in soybean[8]. We have also noticed a severe decline in nodule respiration preceded by an initial increase with developing water stress in several legumes including cowpea (unpublished data).

It is well known that many legumes, including cowpea, transport the fixed nitrogen from nodules to shoot as ureides[13]. In the present study total ureide content of nodules and stem was determined in plants harvested and dried immediately after sampling, as it has been shown that tissue ureide content in cowpea agrees with results of xylem sap analysis for relative abundance of ureides at a given time[14]. In well-watered plants girdling caused an accumulation of ureides in nodules and reduction in ureide level in shoot (table 2). One possible reason for this might be that the stem girdling resulted in deprivation of carbon skeletons needed for the export of ureides from nodules to shoot. However, in stressed plants, girdling did not make any difference in ureide levels. In fact there was a heavy accumulation of ureides in both nodules and shoot. Higher concentrations of tissue ureides in water-stressed plants have been generally observed and are attributed to the low water solubility of ureides as well as to impediments in their further metabolism in shoot[3,9]. Thus, in well-watered plants, stem girdling seems to affect the export of ureides into the shoot, and their accumulation in nodules may possibly inhibit nitrogenase activity. But in stressed plants, any differences in concentration of ureides in relation to stem girdling are not obvious owing to the overwhelming effects of dehydration.

Our results indicate that, in well-watered cowpea plants, a short-term impediment in the translocation of sugars, due to stem girdling, results in a sharp decline in nitrogenase activity by limiting the supply of current photosynthate to the nodules. Carbon skeletons for the export of fixed nitrogen from nodules to the shoot may also be affected. However, when the plants are under water stress, the direct effects of dehydration on nodules become more overwhelming and critical in inhibiting nitrogenase than the photosynthate supply from shoot.

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EFFECT OF STIK ON SPROUTING IN THE STEM CUTTINGS OF *PYRUS PASHIA* BUCH.-HAM. EX D. DON

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THE physiomorphological effect of application of various growth regulators on rooting in stem cuttings has been studied by several workers[1-7]. The growth regulators extensively used by these workers were auxins, especially indoleacetic acid (IAA) and naphthaleneacetic acid (NAA). Though all the auxins have similar qualitative effects, they elicit different responses by the plant owing to differences in their chemical properties[2]. A new growth regulator, Stik (whose active ingredient is NAA as the sodium salt), has also been used and its stimulatory effect on rooting and regeneration of stem cuttings of *Aerva sanguinolenta* has already been proved[8]. This growth regulator has also been shown to stimulate the activity of lenticel meristem in *Sapium insigne*[9].

Stem cuttings of approximately equal diameter (8–10 mm) and length (20 cm) were taken from a healthy *Pyrus pashia* tree growing at an altitude of 1500 m above MSL. Five concentrations, viz. 0.1, 0.2, 0.5, 1.0 and 2.0 mg/ml, of Stik were prepared in distilled water. The cuttings were grouped into batches of 25 for each of the five concentrations and their basal ends were dipped in Stik to a depth of 5 cm for 48 h at room temperature (13 ± 1°C). Control cuttings were dipped in distilled water. All the cuttings were then placed up-side-up in pots containing garden soil and kept under laboratory conditions (9–17°C). The upper ends of the twigs were covered with moist cotton to avoid drying. The experiment was started in the middle of December and the final observations were recorded after five weeks.

Stik has proved to be very potent in inducing sprouting in stem cuttings of *P. pashia*. Although the control set also exhibited sprouting, it was very much less than the sprouting in 0.1 and 0.2 mg/ml of Stik. Further, the adventitious buds produced on cuttings of the control set did not increase much in length. A visible sign of initiation of sprouting was observed first on cuttings treated with 0.1 mg/ml Stik on day 12 of treatment. Cuttings treated with 0.2 and 1 mg/ml Stik showed sprouting on day 15 and those treated with 0.5 and 2 mg/ml on days 17 and 19 respectively. In the control set, sprouting could be observed only on day 22. For all the parameters studied, 0.1 mg/ml was the most potent concentration, followed by 0.2 mg/ml. The leaves were of normal shape and size on cuttings treated with 0.1 and 0.2 mg/ml Stik and on control cuttings (figure 1A–C), but were variously deformed in the other cases (figure 1D–F). In the case of 1 and 2 mg/ml Stik, the leaves became narrower, longer and leathery (figure 1E, F).

The above observations indicate that Stik has a marked influence on adventitious sprouting in *P. pashia*. That the effect is greatest for 0.1 mg/ml Stik is in agreement with the findings of Singh and Paliwal[8], who showed that 100 and 500 ppm (i.e. 0.1 and 0.5 mg/ml) Stik hastened sprouting and subsequent shoot growth in stem cuttings of *Aerva sanguinolenta*. Sharma *et al.*[9] have reported a similar effect of Stik on the lenticel meristem in *Sapium insigne*. Eliason and Areblad[7] reported that IAA at 100 μM usually increased the number of roots, although variable results were obtained with IAA concentrations in light-grown stem cuttings of *Pisum sativum* cv. Weibull's Marina.

As far as the physiomorphological effect is concerned, it has been concluded that the stimulation of sprouting caused by Stik treatment is probably a consequence of conversion of starch to soluble sugars, which thus became available for active cell division and subsequent elongation, as has been postulated by Nanda *et al.*[10] The rapid degradation of starch by auxins is brought about by an increase in the activity of hydrolytic enzymes[11-13]. Altmann and Wareing[14] have also shown that IAA has a definite impact on sugar accumulation; however, according to them the relationship between auxins and carbohydrates is complex, since the auxins may influence basal carbohydrate accumulation directly as well as via the sink arising from the auxin-

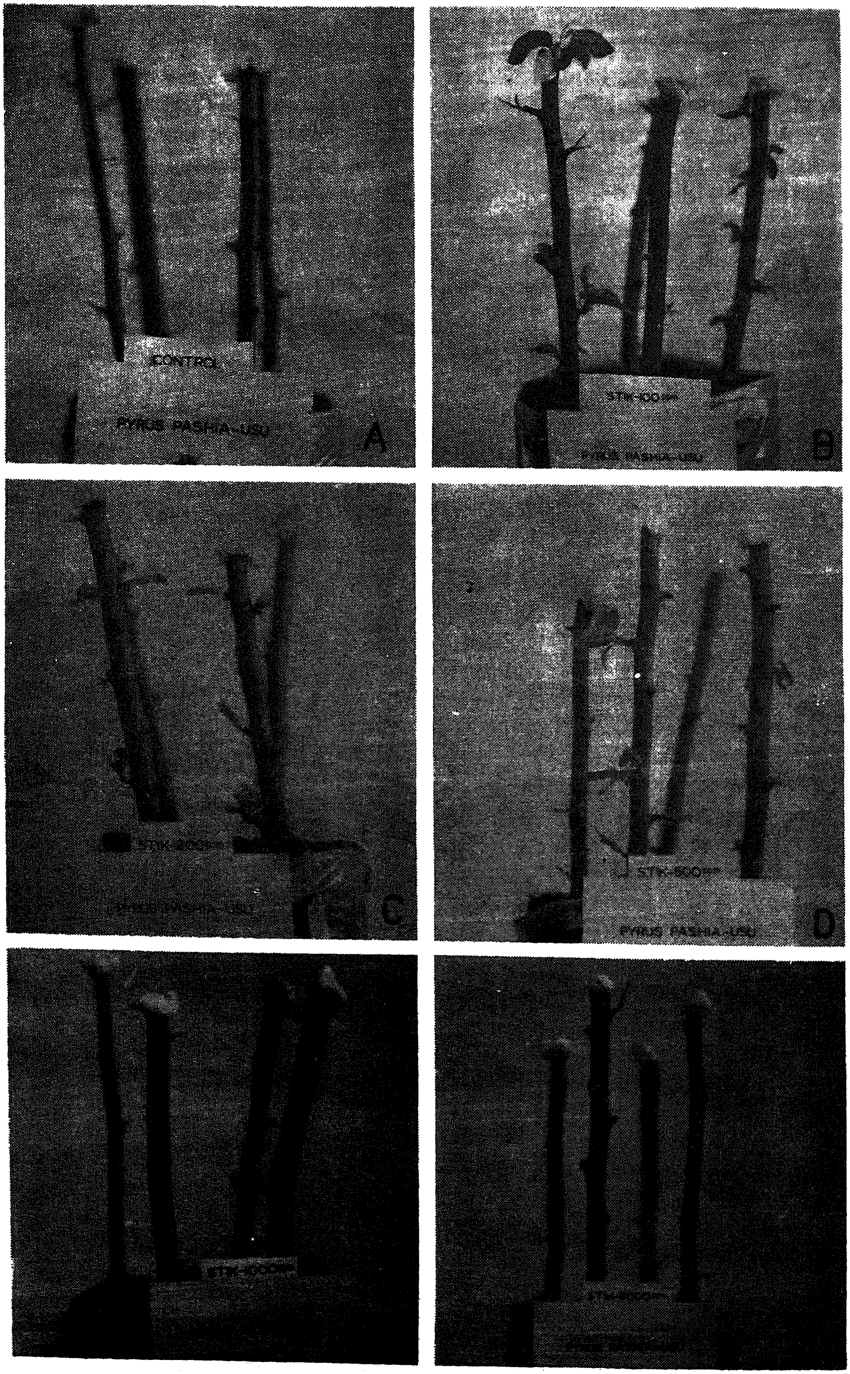

Figure 1. Effect of concentration of Stik on sprouting in stem cuttings of *Pyrus pashia*.

induced rooting. The sprouting induced by the application of Stik in *P. pashia* therefore appears to be mediated by its effect on mobilization of reserve food materials caused by enhanced activity of hydrolytic enzymes. Whatever sprouting was observed in the control set was most probably due to a small amount of endogenous auxin present at the time of cutting.

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NEW EVIDENCE ON THE PHYLOGENY OF BASIC CHROMOSOME NUMBER IN *PENNISETUM*

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THE genus *Pennisetum* (L.) Rich., which belongs to the family Poaceae, has species with chromosome numbers in multiples of $x = 5, 7, 8$ and 9. Of the several economically important species in this genus, pearl millet (*Pennisetum glaucum* (L.) R.Br.) is an important food and fodder crop. Based on chromosome pairing in haploids and interspecific hybrids, Jauhar[1,2] concluded that the chromosome complement of pearl millet ($2n = 14$) was derived from a basic set of $x = 5$ chromosomes. This was contradicted by Manga and Pantulu[3]. The present work on the cytogenetics of wild relatives of pearl millet gives new cytological evidence on the phyletic basic chromosome number and its evolution.

The wild species included in the present study are *P. ramosum* (Hochst.) Schweinf., *P. schweinfurthii* Pilger, and *P. mezianum* Leeke, which were obtained from the wild *Pennisetum* garden maintained by the Genetic Resources Unit, ICRISAT. For cytological studies, young inflorescences were fixed in acetic alcohol (1:3). Iron acetocarmine squash preparations were made from the pollen mother cells (PMC). Photomicrographs of chromosome pairing were taken from the temporary squashes.

Meiosis in *P. ramosum* is normal, with the ($2n = 10$) chromosomes invariably formed into five ring bivalents, one of which is associated with the nucleolus (figure 1A).

Meiosis in *P. schweinfurthii* showed its chromosome number to be $2n = 14$, and the chromosomes paired mostly as seven bivalents. However, of the 60 PMC analysed, 30% showed two trivalents and four bivalents (figure 1B), 45% showed six bivalents and two univalents, 10% had one quadrivalent, four bivalents and two univalents, and the remaining 15% showed seven bivalents. The bivalents are mostly ring bivalents. During anaphase I, most of the cells showed 7:7 normal chromosome distribution. However, a few cells (8%) had 8–6 distribution. The subsequent stages of meiosis were normal. Pollen fertility, as judged by stainability with acetocarmine, was 73%.

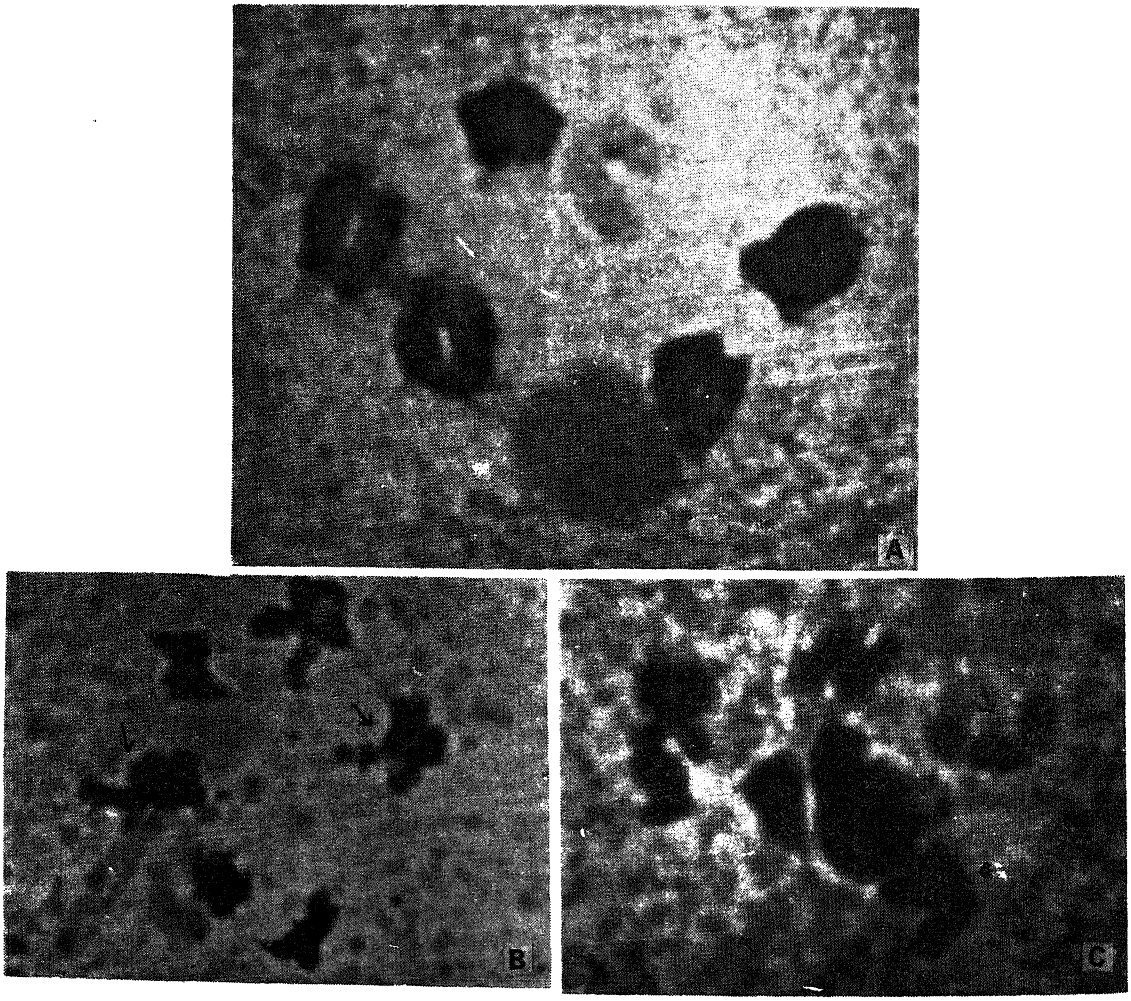

Figure 1A–C. **A,** Diakinesis in *Pennisetum ramosum* ($2n = 10$) showing five bivalents. **B,** Diakinesis in *Pennisetum schweinfurthii* ($2n = 14$) showing two trivalents (arrows) and four bivalents. **C,** Diakinesis in *Pennisetum mezianum* ($2n = 16$) showing two trivalents (arrows) and five bivalents.

P. mezianum showed ($2n = 16$) chromosomes, which were associated mostly as bivalents at diakinesis and metaphase I. Of the 160 PMC analysed, a maximum of two trivalents per cell were observed in 23% of the cells at diakinesis (figure 1C) and 17% in metaphase I. One bivalent was a nucleolar organizer. Various degrees of abnormalities due to these associations were observed in subsequent stages of meiosis. Stainability with acetocarmine indicated that 83% of the pollen grains were normal.

Darlington[4] pointed out that the inference of a basic number in a polyploid series is obviously an important step in fitting chromosome numbers to an evolutionary hypothesis. Sometimes the diploid members of the series have disappeared from the genus. At the same time, most of the 'polyploid' species actually show a diploid-like chromosome behaviour. Phylogenetic divergence in a large number of taxa has occurred, because of increase or decrease from the basic chromosome numbers. Some notable examples are *Frittillaria pudica*[5], where

$x = 13$ has arisen from $x = 12$; Eurasian species of *Allium*[6] ($x = 8$), which originated from the primitive North American species ($x = 7$); and *Zea mays*[7], where $x = 10$ was concluded to be a derivative of $x = 5$. A decrease in basic chromosome number has also been observed in many plants[8].

Formation of two trivalents per cell in 23% of cells of *P. mezianum* and 30% of cells of *P. schweinfurthii* suggests duplication of two chromosomes in these species. Formation of one quadrivalent in *P. schweinfurthii* was also reported earlier[9]. The occurrence of higher associations in these species could be attributed to homology to the basic complement following duplication and differentiation during the evolution of these species.

Based on the observed higher associations in the diploid complement of *P. schweinfurthii* and *P. mezianum*, it may be assumed that the chromosome complement in *Pennisetum* has evolved from a basic chromosome number like $x = 5$. The occurrence of a species with $x = 5$ in *Pennisetum*, such as *P. ramosum*, further supports this view. This is in agreement with the conclusions of Jauhar[2] and suggests that *P. glaucum* is a secondary balanced species with phyletic basic chromosome number $x = 5$.

Thanks are due to Mr C. Rajagopal Reddy, ICRISAT, for assistance in maintaining the wild species.

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INDUCED COMPLEX TRANSLOCATION HETEROZYGOSITY IN CHILLI

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IN work on induced mutagenesis in chillies, a complete sterile plant was isolated from the M_1 population of a fast-neutron treatment (2.6×10^{10} n/cm^2/4.5 min). As there was poor flowering and no fruit setting in the plant, its meiosis was studied.

Genetically pure and 'dry' seeds (moisture content 6.08%) of *Capsicum annuum* L. cv. NP 46A were subjected to fast neutron treatment at the Bhabha Atomic Research Centre, Trombay. The M_1 population was raised from the irradiated seeds along with control plants. Flower buds of suitable size were fixed in acetic: alcohol (1:3) and squashed in 1% acetocarmine solution.

The sterile plant differed from the control in several morphological characters (table 1). It was characterized by delayed flowering. No fruit set was obtained because of cent per cent flower abscission.

Meiotic characters of the control and sterile plants are given in table 2. Control plants showed 12 bivalents uniformly at diakinesis (figure 1). In the aberrant type, a hexavalent ring or chain was commonly found in many of 200 pollen mother cells (PMC) studied; the ring configuration was most common (figure 2). Only terminal chiasmata were observed in both the control and the aberrant type. The meiotic abnormalities included unoriented chromosomes (14.98% at MI, 2.34% at MII), laggards (11.26% at AI, 2.81% at AII), unequal distributions (4.69% at AI, 1.87% at AII) and chromosome bridges (10.32% at AI). Total meiotic abnormalities (46.47%) and pollen viability (23.15%) in the sterile

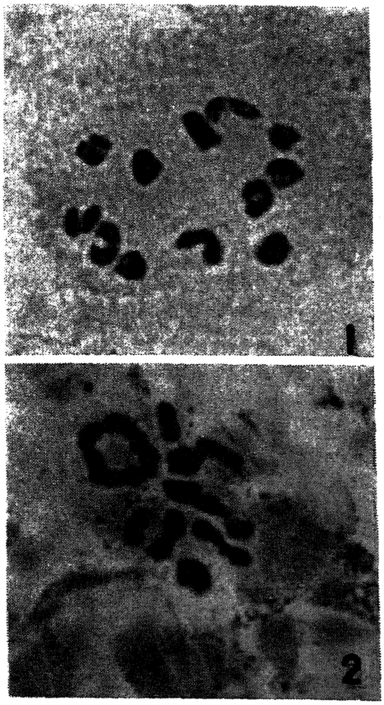
Table 1 *Comparative morphology of control and sterile Capsicum annuum*

Plant character	Control	Sterile
Plant height (cm)	38.0	27.0
Plant spread (cm)	52.0	20.0
No. of main branches	1	2
Leaf size (l × b of lamina, cm)	4.7 × 3.2	2.2 × 1.8
Petiole length (cm)	2.4	1.1
Days to flowering	80	110
Total no. of flowers formed	200	25
Flower abscission (%)	22	100
Total no. of fruits formed	178	—

Table 2 *Meiotic characters of control and sterile Capsicum annuum*

Meiotic character	Control	Sterile
Univalents	0 (0)	0–5 (0.23)
Ring bivalents	1–6 (2.88)	0–5 (0.29)
Rod bivalents	6–11 (9.12)	2–11 (8.25)
Ring hexavalent	0 (0)	0–1 (0.7)
Chain hexavalent	0 (0)	0–1 (0.16)
Average chiasma frequency/cell	14.88	13.92
Average chiasma frequency/ chromosome	0.62	0.58
Meiotic abnormality (%)	8.33	46.47*
Pollen viability (%)	88.54	23.15*

Figures in parentheses are average.
*Significant at 1% level.



Figures 1 and 2. **1,** Diakinesis in control plant showing 12 bivalents. **2,** Diakinesis in sterile plant showing one hexavalent and nine bivalents.

plant were significantly different (at 1% level) from those in the control.

Translocation heterozygotes involving quadrivalents were reported earlier in chilli from fast neutron and gamma ray-treated populations[1, 2]. Since hexavalent configuration commonly appeared in PMC of the sterile plant, it indicates a case of complex translocation heterozygosity. In complex heterozygotes, two reciprocal translocations are probably involved in causing six chromosomes to unite in a pairing configuration. The complex heterozygosity is well known in the genus *Oenothera*, where not only forms with ring of 6 chromosomes are present but also those with ring or chains of 8, 10, 12 and even all 14 chromosomes[3]. Multiples with chromosome association greater than four are reported to occur naturally in some plants like *Clarkia*, *Paeonia*, etc[4].

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TISSUE CULTURE STUDIES ON DESERT PLANTS: I. *CENCHRUS CILIARIS* CV. 75

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PLANT cell and tissue culture offer great potential in evolving elite plants for sub-optimal environments. Our research programme, using this method, is concerned with improvement of fodder grasses of the Thar desert with respect to their tolerance to drought, salt, heavy metals and other environmental constraints. In this communication we report conditions that favour *in vitro* differentiation of plantlets from young inflorescences of *Cenchrus ciliaris* (Anjan Dhaman) cv. 75.

Young inflorescences, before their emergence from the sheathing leaves, were removed from the plants and surface-sterilized by thoroughly wiping with 70% ethanol and then treating them with mercuric

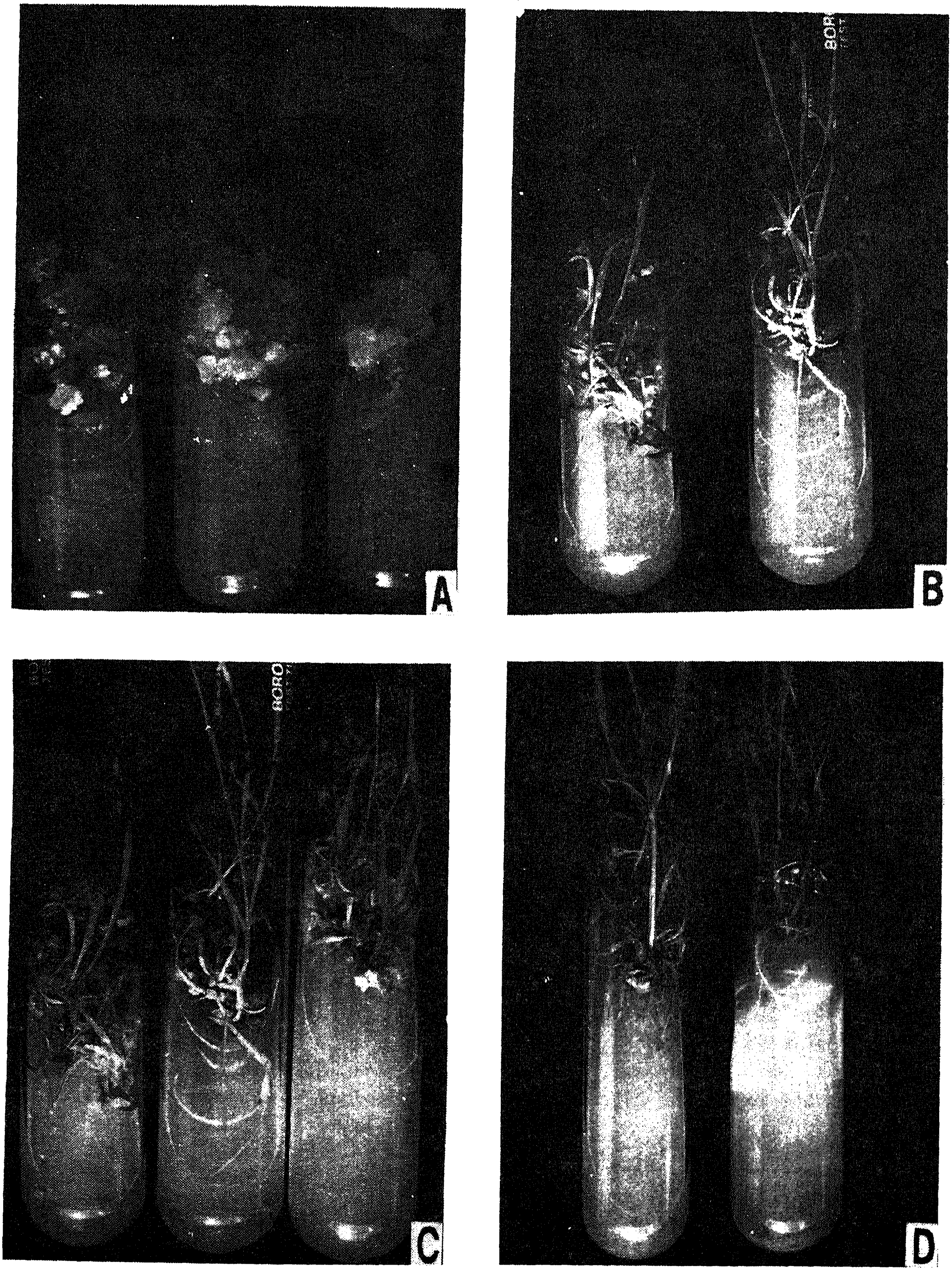

Figure 1A–D. Different stages of plantlet regeneration from inflorescence cultures of *Cenchrus ciliaris*. **A,** Callus cultures on MS + 2,4-D (1 mg/l) + IAA (5 mg/l) + kinetin (0.5 mg/l). **B and C,** Plantlets growing on MS medium devoid of growth regulators. **D,** Regenerants with well-developed roots.

chloride (0.2%) for 5 min. After a thorough wash with autoclaved distilled water the sheaths were removed and inflorescence segments measuring about 1 cm were inoculated on callusing medium containing Murashige and Skoog's (MS)[1] nutrients supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 1 mg/l), 3-indoleacetic acid (IAA, 5 mg/l) and kinetin (0.5 mg/l). The cultures were incubated under a 16-h photoperiod at 25 ± 2°C.

Callus initiation started within 4–6 days and good proliferating calli were derived by the end of 21 days. Nearly all parts of the inflorescence segments, except the glumes, showed proliferation. The calli produced were white, soft, compact and slow-growing. Most of the cultures had generated white, hard structures on the surface at the end of 10 weeks (figure 1A).

Subcultures were usually carried out every 20–24 days on the same medium. After 12–14 weeks of growth the calli showed numerous dome-shaped, greenish, localized areas from which green, leafy shoot buds developed in due course of time. Often the green areas were found to be flanked by green, leafy structures, which were actually the enlarged scutella of the precociously germinating embryos[2-4]. The frequency of shoot bud formation ranged between 5 and 9 per culture. Further development of shoot buds was achieved after transferring them to a medium devoid of growth regulators (figure 1B, C). On this medium, the cultures with shoot buds showed profuse rooting. The majority of the cultures developed several roots (figure 1C, D). Of these, some roots indicated direct link with the shoots. These *in vitro* produced plantlets were successfully transferred to soil, and up to 60% of the plantlets survived to maturity.

Financial support from UGC, New Delhi, is gratefully acknowledged.

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RECOVERY OF RARE INTERSPECIFIC HYBRIDS OF GRAM *CICER ARIETINUM* × *C. CUNEATUM* L. THROUGH TISSUE CULTURE

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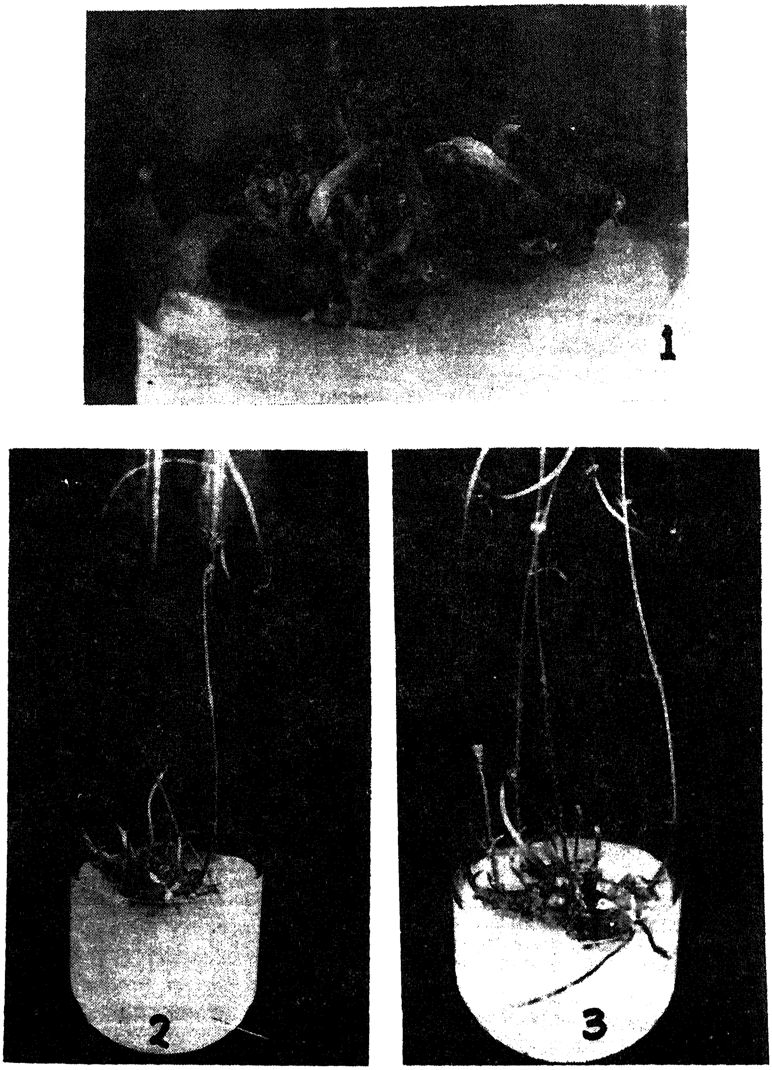
THE genus *Cicer* has a total of 43 species of which only one, *Cicer arietinum* L., is cultivated while the rest are wild. *C. arietinum* (chickpea) crosses readily with only *C. reticulatum*. Attempts to hybridize it with other wild *Cicer* species have so far been unsuccessful[1]. Bridge hybridization among wild species was also not successful[2]. This has seriously hampered utilization of germplasm from wild relatives of chickpea in breeding programmes. Embryo rescue is a powerful technique to obtain hybrid plants from sexually incompatible crosses. Several rare hybrids have been obtained from the crosses of sexually incompatible species using the embryo culture technique[3]. An attempt was therefore made to cross chickpea with *C. cuneatum*.

More than 2000 crosses were made but no seed-bearing pods were obtained. Manipulations like removal of stigma and part of style, pollination at bud stage, and delayed pollination were not beneficial[2,4]. However, in one cross ten flowers were retained on the plant up to the 5th day. Generally, pollinated flowers dropped 4–5 days after pollination. Bassari *et al.*[4] suggested that interspecific hybrids could be obtained through embryo culture from incompatible crosses of *Cicer* species. Hence, the ten pollinated flowers retained on the plant till the 5th day were surface-sterilized with 8% calcium hypochlorite for 15 min and washed with sterile distilled water 5–6 times. The ovaries of these flowers were dissected under a dissecting microscope and transferred to culture tubes containing embryo regenerating medium, conditioned with chickpea embryo culture (figure 1, B_5 basal medium[5] containing 0.5 mg/l BA, 0.1 mg/l naphthaleneacetic acid (NAA) and 100 ml/l coconut milk). The cultures were maintained at 26 ± 2°C under a cool fluorescent light (4000 lux) in a 12-h photoperiod.

Eight of the cultures showed callus formation while the other two showed shoot-bud formation accompanied by small amount of callus. These shoot buds produced 10–15 shoots in each culture tube after 8–10 days (figure 2). Each of these shoots with small amount of callus, on further transfer to the



Figures 1–3. **1,** Multiple shoot formation from young embryo (8-day-old) of chickpea. **2,** Regenerated shoots from shoot bud developed from fertilized ovule of the cross *C. arietinum* × *C. cuneatum*. **3,** Complete plantlet regeneration.

same medium in culture tubes, produced many shoots/plantlets within 20 days. In most of the cases shoots were accompanied by roots. Profuse root regeneration was achieved on transferring the shoots to B_5 medium containing 1 mg/l NAA alone. The plantlets were transferred to small plastic cups, with 40% success. The surviving plants were transferred to pots. The hybrid plants were morphologically more similar to *C. cuneatum*, showing small linear leaflets (figure 3), although chickpea (*C. arietinum*) was the female parent. This confirms that the regenerated plants are hybrid and not selfed plants; selfed plants would be similar to chickpea morphologically. The hybrid plants did not flower.

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IN VITRO MASS-SCALE PROPAGATION OF *ROSA HYBRIDA* CV. LANDORA

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CONVENTIONALLY, most of the garden roses are propagated by budding or grafting of the scions on to suitable rootstocks. Of late, tissue culture techniques have been applied for rapid propagation of many hybrid rose cultivars[1] and it has been suggested that roses can be propagated through tissue culture on a commercial scale and can be grown successfully on their own roots[2,3]. It has also been stressed that tissue culture practices offer the possibility of eliminating pathogens from infected stocks, and also reduce the time and financial inputs for propagation[3]. In this investigation tissue culture protocols for mass-scale propagation of *Rosa hybrida* cv. Landora have been standardized. This cultivar shows considerably poor multiplication rate in conventional methods.

Young branches with dormant axillary buds were cut from field-grown plants of *Rosa hybrida* cv. Landora and brought to the laboratory with their cut ends dipped in distilled water. The terminal portion with 5–6 nodes of each branch was discarded and the subsequent 10–12 nodes were taken for culture. The axillary buds with a small part of the stem (~2 mm) on both sides were cut off and surface-sterilized in 0.1% mercuric chloride solution for 25 min. The sterilized materials were rinsed four times in sterile distilled water and aseptically inoculated on to solidified MS medium[4]. The basal medium was supplemented with different concentrations of 6-benzylaminopurine (BAP), gibberellic acid (GA_3), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) singly or in combinations, and 30 g/l of sucrose, and gelled with 8 g/l of agar (Glaxo, India). The pH of the medium was adjusted to 5.8 and the medium autoclaved for 20 min at 121°C and 1.06 kg/cm^2. All cultures were raised in 250 ml Erlenmeyer flasks (Borosil, India) and incubated at 25 ± 2°C under 14-h photoperiod provided by cool white fluorescent light (ca. 3000 lux). For induction of rooting microshoots were cultured on a filter paper bridge placed in liquid medium.

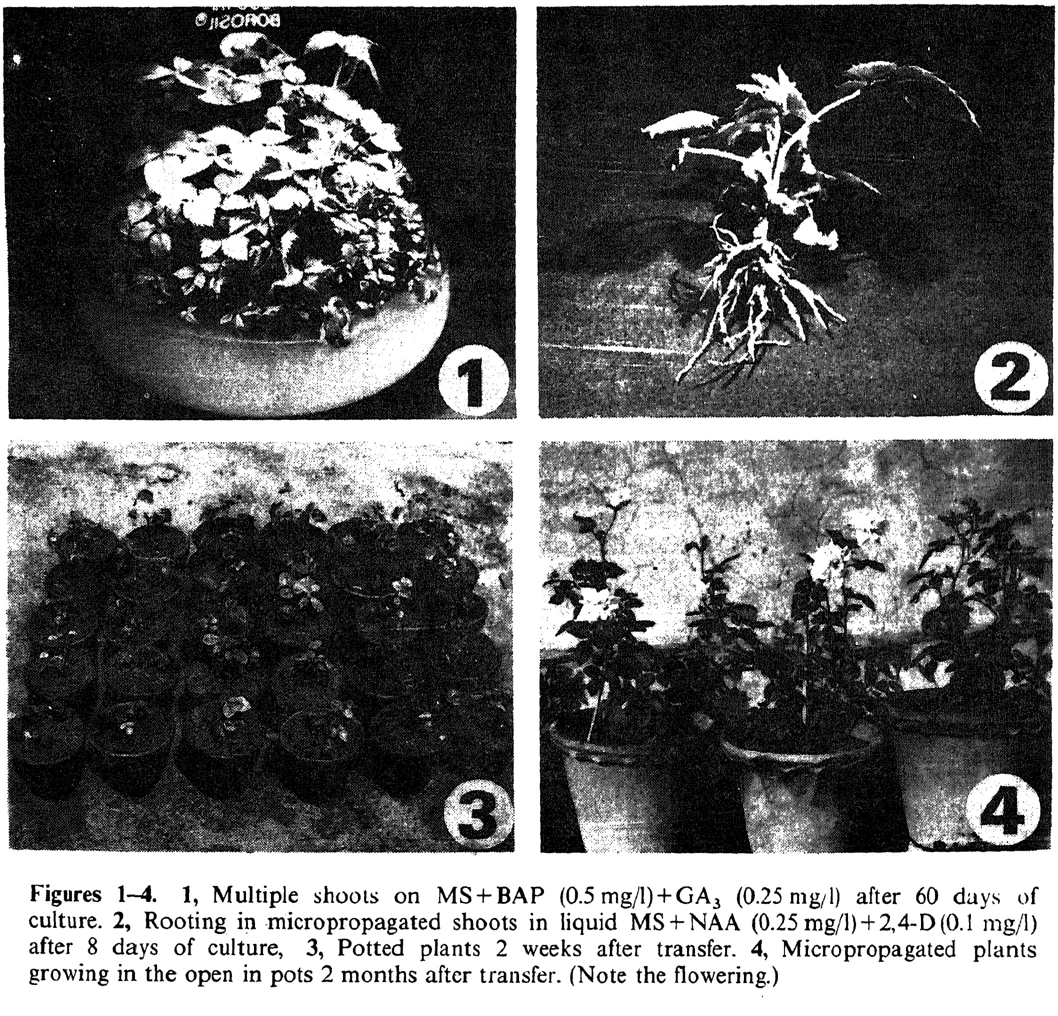
On a hormone-free MS medium the explants showed bud-break after about 22–30 days and the buds elongated a little but the subsequent growth of the explants was totally supressed. In BAP-supplemented, or (BAP and GA_3)-supplemented media, early bud-break in about 6–10 days was noticed and multiple shoots were formed subsequently (table 1). Addition of BAP (0.25–1.0 mg/l) alone to the medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably slow, though the average number of shoots produced in a 60-day culture period was around 4 per explant. Explant response remained between 63 and 80%. Incorporation of GA_3 at low concentrations (0.25–0.5 mg/l) in the BAP-supplemented medium gave encouraging results:

Table 1 *Induction of multiple shoots from axillary buds of Rosa hybrida cv. Landora cultured on MS medium with BAP and GA_3 after 60 days*

Treatment (mg/l)	No. of explants cultured	Cultures with multiple shoots (%)	No. of shoots per explant (mean ± SE)
—	126	—	1
BAP (0.25)	252	71.4	4.08 ± 0.75
BAP (0.5)	240	80.0	4.00 ± 0.08
BAP (1.0)	276	63.0	4.33 ± 0.17
BAP (0.25) + GA_3 (0.25)	220	95.0	3.25 ± 0.59
BAP (0.25) + GA_3 (0.5)	240	91.6	2.66 ± 0.84
BAP (0.5) + GA_3 (0.25)	240	95.0	5.25 ± 0.92
BAP (0.5) + GA_3 (0.5)	220	90.0	4.75 ± 0.92

explant response improved to 90–95% and the number of shoots produced per explant was highest (5.25±0.92 shoots/explant) when the medium contained 0.5 mg/l of BAP and 0.25 mg/l of GA_3 (figure 1). When the shoots were separated from the initial culture and recultured in a medium with the same composition the average number of shoots produced per explant remained more or less the same as that in the first passage. Thus the multiplication after two passages, each of 60 days, was about 25 shoots for each explant cultured initially in the first passage. Shoots were separated from the clumps and cultured in liquid MS medium supplemented with NAA (0.25–1.0 mg/l) and/or 2,4-D (0.1–1.0 mg/l) for rooting (table 2). Shoots cultured in medium containing NAA or 2,4-D above 0.5 mg/l or 0.5–1.0 mg/l of NAA with 0.1 mg/l of 2,4-D rooted in about 12 to 14 days but with basal callusing. However, the best rooting (~6 roots/shoot) was observed in about 8 days in medium with the combination 0.25 mg/l of NAA and 0.1 mg/l of 2,4-D. These roots elongated in a further period of one week (figure 2). The micropropagated plants were then put into earthen pots (figure 3) containing a mixture of sand, soil and cow-dung manure (1:1:1). After hardening for two weeks under a polythene tent maintained at 25–30°C and 60–70% relative humidity the plants were exposed to the environment. The plants could be grown successfully to flowering in the field (figure 4).

The present results indicate that, starting with a single axillary bud of *Rosa hybrida* cv. Landora as explant, it is possible to obtain about 25 plants after 120 days of culture, and these micropropagated plants can be grown in the open with very low



Figures 1–4. 1, Multiple shoots on MS+BAP (0.5 mg/l)+GA_3 (0.25 mg/l) after 60 days of culture. **2**, Rooting in micropropagated shoots in liquid MS+NAA (0.25 mg/l)+2,4-D (0.1 mg/l) after 8 days of culture, **3**, Potted plants 2 weeks after transfer. **4**, Micropropagated plants growing in the open in pots 2 months after transfer. (Note the flowering.)

Table 2 *Effect of NAA and 2,4-D on rooting in micropropagated shoots of Rosa hybrida cv. Landora cultured in liquid MS medium*

Treatment (mg/l)	No. of shoots cultured	Shoots rooting (%)	Days to rooting	No. of roots per shoot (mean ± SE)
—	152	—	—	—
NAA (0.25)	176	63.6	14	3.66 ± 0.57
NAA (0.5)	160	80.0	12	3.88 ± 0.73
NAA (1.0)	154	81.8	12	2.58 ± 0.59*
2,4-D (0.25)	168	90.0	12	3.20 ± 0.26
2,4-D (0.5)	154	80.0	12	3.50 ± 0.56
2,4-D (1.0)	160	50.0	14	2.00 ± 0.62*
NAA (0.25) + 2,4-D (0.1)	154	90.9	8	6.00 ± 0.81
NAA (0.5) + 2,4-D (0.1)	140	80.0	10	5.10 ± 0.83*
NAA (1.0) + 2,4-D (0.1)	176	54.5	12	2.66 ± 0.69*

*Callusing at the base of shoots.

mortality. Similar results have been reported from micropropagation experiments in some difficult-to-propagate rose cultivars[3,5-8]. Roses being very popular as garden plants throughout the world, any such practice that can accelerate the propagation rate should be of high commercial value to the rose industry. It seems conceivable that in future many important cultivars of roses would be commercially propagated through tissue culture[3].

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DIABETOGENIC NATURE OF OCHRATOXIN A

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OCHRATOXIN A, a hepatotoxic mycotoxin, is produced as a secondary metabolite by *Aspergillus ochraceus* and *Penicillium virridicatum*[1]. Ochratoxin A ingestion causes increase in blood glucose concentration[2]. *A. ochraceus* has been reported to grow on a wide variety of foodstuffs and feeds, including rice, wheat, corn and peanuts[3]. Ochratoxins and ochratoxin-producing fungi have been isolated and purified from food samples meant for human consumption in various parts of the world. Previous reports reveal that *A. ochraceus* contamination is very frequent in India, especially in the southern region, which is evident from the reported isolation and identification of ochratoxins in some warehouse samples in Tamil Nadu and Hyderabad[4,5]. Ingestion of mold-contaminated diet poses health hazards to human beings. This communication reports results of a study on the effect of ochratoxin A on liver carbohydrate, blood glucose and serum insulin levels.

The strain of *A. ochraceus* used was isolated in our laboratory from fungus-contaminated feed. The identification was confirmed by the Indian Agricultural Research Institute, New Delhi, India. The strain was found to produce ochratoxin A as a major secondary metabolite. Ochratoxin A was isolated from the culture filtrate by the method of Suzuki *et al.*[6] An authentic sample of ochratoxin A supplied by Dr Timothy D. Phillips, Department of Veterinary Public Health, Texas A & M University, USA, was used as reference.

Weanling albino rats from our laboratory animal colony weighing 80 ± 15 g were used. The rats were divided into two groups, each comprising 15 rats. One group was administered orally with 0.5 ml of 0.1 M sodium bicarbonate solution[7] containing 100 μg of ochratoxin A per rat per day and the other group was maintained as control.

After eight weeks blood was collected from the tail of the rats and blood glucose concentration was estimated[8]. Since the fasting blood glucose concentration of the experimental group of rats was elevated, tolerance to glucose was tested. The rats were starved overnight and fasting blood samples were collected. Glucose solution (10%) was administered orally (3.5 g per kg body weight). Blood samples were collected thereafter over a period of 120 min every 30 min. One set of blood samples was collected with sodium fluoride as anticoagulant for glucose estimation and another set was collected for the assay of serum insulin by the method of Herbert *et al.*[9] Later the animals were killed and liver tissue was used for the estimation of total carbohydrates and glycogen[10].

The blood glucose and serum insulin levels of control and ochratoxin A-administered rats are given in table 1. The blood glucose and serum insulin levels during the glucose tolerance test are shown in figures 1 and 2 respectively. In control rats maximum blood glucose concentration was reached 60 min after glucose load and the concentration dropped back to fasting level by 120 min. But in the treated rats blood glucose concentration (maximum around 60 min) did not return to fasting level even at the end of 120 min, indicating an imbalance in glucose homeostasis during ochratoxin A toxicosis (figure 1). Though insulin level increased immediately after glucose load, the treated rats showed insufficient increase in insulin level (figure 2) to utilize the ingested glucose, which resulted in hyperglycemia. Total carbohydrates and glycogen were reduced in liver tissue of treated rats (table 2).

Table 1 *Blood glucose and serum insulin levels in control and ochratoxin A-administered rats*

Parameter	Control	Ochratoxin A-administered
Blood glucose (mg/100 ml)	59.20 ± 4.80	112.40 ± 5.10*
Serum insulin (μIU/ml)	21.30 ± 1.30	14.50 ± 1.80*

Values are mean of six observations.
*Statistical significance, $P < 0.001$.

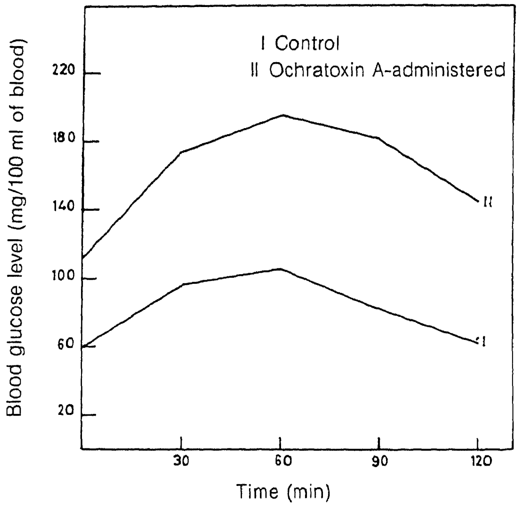

Figure 1. Blood glucose levels in control and ochratoxin A-administered rats during glucose tolerance test.

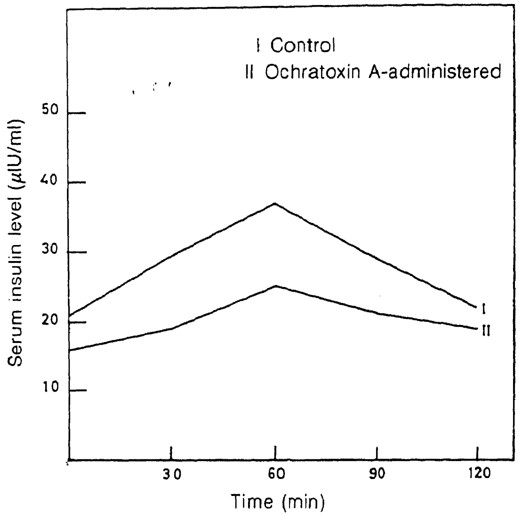

Figure 2. Serum insulin levels in control and ochratoxin A-administered rats during glucose tolerance test.

Normally blood glucose level is maintained by the regulatory pancreatic hormones insulin and glucagon. Insulin and glucagon influence carbohydrate metabolism in an antagonistic manner. However, utiliza-

glycogen in control
rats

[illegible]	Ochratoxin A-administered
[illegible]	39.22 ± 4.76*
[illegible]	20.50 ± 3.90*

fresh tissue and are

mainly controlled by the
is the best inducer of
islets of pancreas. The
during ochratoxin A
decreased level of insulin,
to inhibited synthesis
insulin from the pancreatic
associated with
like terreic acid[11]
reported. Enhanced
by depletion of hepatic
ochratoxin A toxicosis has

'glucostat' and plays a
of blood glucose level
release of glucose into
A has been reported to cause
It is important to note that
in the level of total
during ochratoxin A
of glycogen decrease.
in the level of glycogen may
ochratoxin A on glycogen
like aflatoxin[15] and
reported to lower hepatic

A administered rats exhibited a
it may be concluded that
secondary metabolite of
in nature.

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CHROMOSOMAL STUDIES IN *FULVIA MUTICA* (BIVALVIA: CARDIIDAE) FROM JAPAN

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CHROMOSOMAL studies on a large number of molluscs belonging to different families were made earlier[1-4]. Nakamura[2] listed cytogenetic information on 125 species of molluscs using the CISMOCH computerized index system. However, in family Cardiidae only five species were recorded. The present report of chromosome number and karyotype

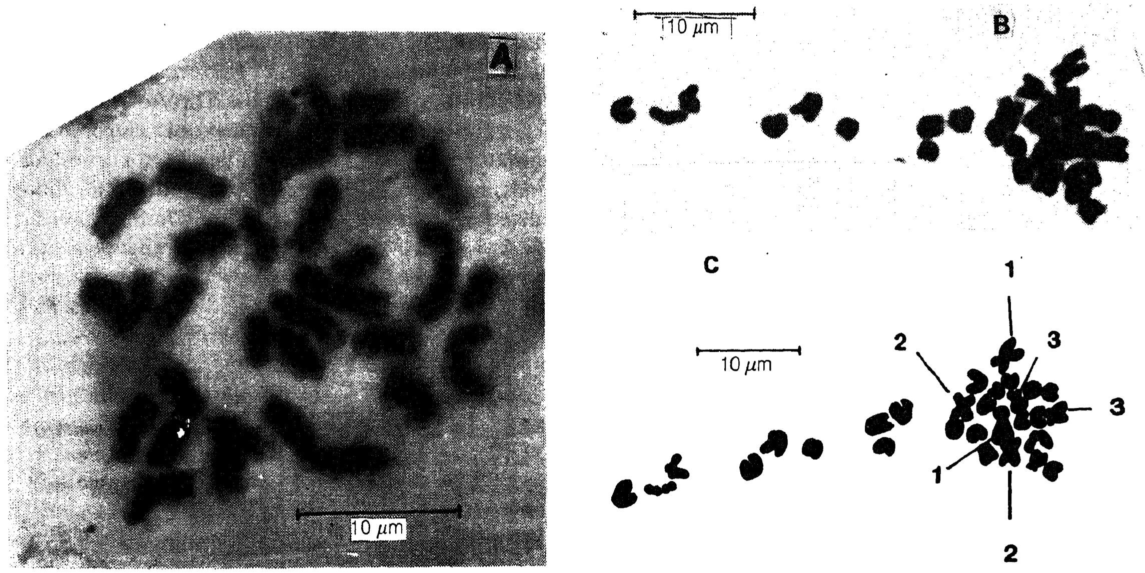

Figure 1A–C. Chromosomes of *Fulvia mutica.* **A,** Late prophase cell showing chromosome number (2*n*) of 30. **B,** Somatic metaphase. **C,** Karyotype.

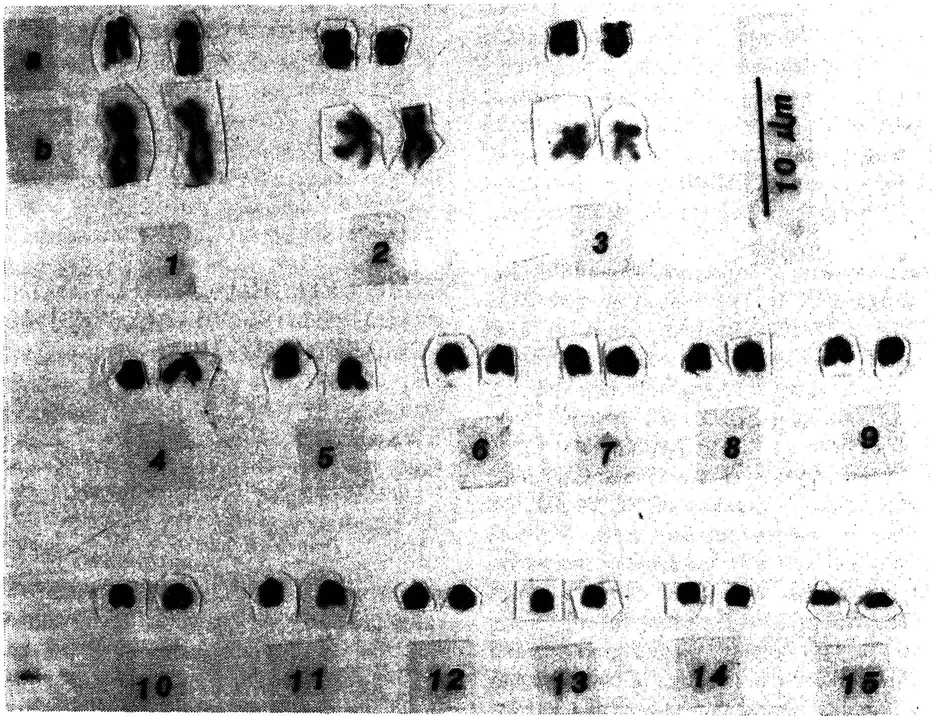

Figure 1D. Karyogram from figure 1A. (The pairs 1–3 were, respectively, submetacentric, metacentric and submetacentric. The pairs in line B from a late prophase cell are included to show clearly the position of the centromere.)

Table 1 *Karyotypic data for Fulvia mutica*

Chromosome pair	Total length (μm) Mean	SD	Arm ratio (L/S) Mean	SD	Chromosome type*
1	4.75	0.82	1.83	0.31	SM
2	3.85	0.93	1.08	0.31	M
3	2.75	0.87	2.03	0.45	SM
4	2.27	0.84	4.50	0.78	ST
5	2.06	0.69	—		T
6	1.96	0.75	—		T
7	1.84	0.52	—		T
8	1.82	0.46	—		T
9	1.80	0.50	—		T
10	1.75	0.25	—		T
11	1.72	0.31	—		T
12	1.64	0.29	—		T
13	1.58	0.25	—		T
14	1.52	0.45	—		T
15	1.20	0.35	—		T

*SM, Submetacentric; M, metacentric; ST, subtelocentric; T, telocentric.
Karyotype formula: 4 SM + 2 M + 2 ST + 22 T.

of *Fulvia mutica* Reeve (1844) is a new addition to the existing data on the family.

Thirty specimens of 6-month-old *F. mutica*, reared at Kyoto Prefecture Fisheries Station, Japan, were collected in April 1988, and chromosomal studies were carried out in gill tissue cells. The gills were dissected, treated with 0.04% colchicine (30 min), and fixed in Carnoy's (3:1). Slides were prepared by the air-drying technique and stained with Giemsa stain.

The chromosome number ($2n$) was found to be 30, as shown in figure 1A–D. The karyotypic data were obtained from ten metaphase plates (table 1). The somatic diploid complement comprises 4 submetacentric, 2 metacentric, 2 subtelocentric and 22 telocentric chromosomes (figure 1D). The chromosome size varies between 1.20 and 4.75 μm.

Among the five species of the family Cardiidae studied earlier, diploid numbers of 38, 38 and 24 were recorded in *Mercenaria mercenaria*, *M. campechiensis* and *Dinocardium robustum* respectively, and $n = 20$ in *Cardium edule* and *Cardium tuberculum*[1-3]. The results of the present investigations on *F. mutica* ($2n = 30$) suggest that the species in the family Cardiidae have a wide range of chromosome number. Nakamura[2] found that more than half of the diploid complements of bivalves were usually metacentric and submetacentric, except in family Mytilidae, Pectinidae and Terenidae. The present information on *Fulvia mutica* (Cardiidae) shows dominance of telocentric chromosomes in the diploid complement.

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RAPID DETECTION OF AFLATOXIN BY PRESSURE MINI-COLUMN TECHNIQUE: A COLLABORATIVE TRIAL

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AFLATOXINS are toxic secondary metabolites elaborated primarily by *Aspergillus flavus* and *A. parasiticus*. They are commonly found to contaminate diverse agricultural commodities consumed by man, thus posing a potential health hazard[1,2]. An outbreak of acute toxic hepatitis in India, due to consumption of maize heavily contaminated with *A. flavus*, was reported from tribal areas in Rajasthan and Gujarat[3,4]. A similar outbreak was subsequently reported from Kenya[5]. Epidemiological studies have shown an association between dietary aflatoxin exposure and the high incidence of primary liver carcinoma[6,7]. Aflatoxins have also been implicated in other human diseases—kwashiorkor in children in Sudan[8], Reyes syndrome[9], and Indian childhood cirrhosis[10]. There is therefore growing concern about aflatoxin contamination in foods and feeds. Recently the Government of India fixed the tolerance limit for aflatoxins at 30 ng/g for certain agricultural commodities under the Prevention of Food Adulteration Act. The Codex Alimentarius Commission of the FAO/WHO has suggested fixing a limit of 15 ng/g for aflatoxin in nuts and cereals.

Various analytical techniques, viz. thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), etc., have been developed for the detection of aflatoxins in foods and feeds. Most of these methods require sophisticated equipment and elaborate clean-up of the sample before analysis for aflatoxins, and are thus not useful for routine screening for and detection of aflatoxins in agricultural commodities. One such method is mini-column chromatography[11,12]. The mini-column has been further modified, and a simple, rapid and reliable method for detection of aflatoxin in foods and feeds has been developed[13]. This technique employs commonly available glass syringes tightly packed with adsorbants. The syringes also allow application of positive pressure, which helps in better separation of aflatoxins.

The pressure mini-column method would be useful for the public health analytical laboratories of the State Governments, which have to mandatorily analyse samples of agricultural commodities suspected to be contaminated with aflatoxins. Sometimes the results obtained in the Central Appellate Laboratories are at variance with those of State Analytical Laboratories. In order to minimize such errors among the various analytical laboratories it was considered essential to have a uniform procedure and find the extent of variation in the results. A study was therefore designed to collaboratively evaluate the method, with regard to its practical application and the extent of variation in results from analysts of different public health analytical laboratories.

Samples: Naturally contaminated maize samples containing aflatoxins were ground and blended. The ground maize was divided into sample I and sample II, which served as aflatoxin-positive samples. A third sample (sample III), of aflatoxin-free maize, was used as control sample. Each of the three samples was given a code number. Each analyst received three samples, which were picked up randomly from two lots, containing contaminated maize and control maize samples. Pre-packed pressure mini-columns and aflatoxin standards were supplied to the analysts for the analysis of the sample.

Chemicals: Five-ml glass syringes of borosilicate glass (11 mm i.d.) obtained from Hindustan Syringes, India, Florisil (100–200 mesh) from Sigma Chemical Co., USA, column grade silica gel (60–120 mesh) from Acme Synthetic Chemicals, India, and neutral alumina (active) from BDH Chemicals, India, were used in the study. Aflatoxin B_1 standard was obtained from Roth Chemicals, West Germany.

Preparation of pressure mini-column: A glass syringe of 5 ml capacity was taken and a filter paper disc (Whatman No. 1) was placed at the bottom. The syringe was packed in the following order: finely powdered anhydrous sodium sulphate (3 mm), silica gel (5 mm), Florisil (1 mm), filter paper disc, anhydrous sodium sulphate (4 mm), followed by a filter paper disc at the top. Each layer was tightly packed using a plunger of diameter smaller than that of the syringe. The column was then activated at 110°C for 1–2 h.

Sample preparation: *Step 1*: To 10 g of sample powder in a 250 ml conical flask, 50 ml of acetone: water (85:15) and 2.5 g of celite were added and the mixture shaken for 30 min on a wrist-arm shaker.

Step 2: The extract was filtered through Whatman No. 1 filter paper. Ten ml of the filtrate was taken into a test tube and 10 ml of 20% lead acetate was added and the two mixed vigorously for 3 to 4 min and then filtered through Whatman No. 1 filter paper.

Step 3: Two ml of benzene was added to the filtrate of step 2 and mixed thoroughly to extract aflatoxins into the benzene layer in a 50 ml separating funnel. The lower layer was discarded and the upper benzene layer was collected in a test tube. To the benzene extract 400 mg of neutral alumina (active) was added. The extract and alumina were mixed for 20 min and allowed to stand.

Step 4: One ml of the clear benzene layer from

Table 1 *Results of analysis of aflatoxin-contaminated (I and II) and uncontaminated (III) samples by ten analysts.*

Aflatoxin concentration (ng/g)	Sample I		Sample II		Sample III	
	Positive	Negative	Positive	Negative	Positive	Negative
< 60	—	—	(1)	—	—	—
60–120	(10)	—	(8)	—	—	—
>120	—	—	(1)	—	—	—
Not detected	—	—	—	—	—	(10)
Total	(10)		(10)			(10)

Numbers in parentheses are number of analysts.

step 3 was slowly loaded onto the activated pressure mini-column and the benzene layer was allowed to enter the column by gravity. Three ml of cleaning solvent (hexane:chloroform:tetrahydrofuran, 7:2:1) was immediately added to the column and rapidly drained by applying pressure with the plunger.

Step 5: The pressure mini-column developed as above was observed under long-wave UV light for the presence of bright blue fluorescence. This fluorescence was visually compared with the fluorescence of the standard aflatoxin B_1 provided (30 ng/ml, 60 ng/ml and 120 ng/ml). The values were reported to the nearest standard or a standard range on a printed data sheet. All the collaborators were given a trial demonstration of the procedure before the actual analysis of samples. The maize samples were also analysed at the National Institute of Nutrition by the pressure mini-column and HPLC methods[13,14].

The results are presented in table 1. The maize samples supplied to the analysts were also analysed at this Institute by the pressure mini-column technique and by HPLC. The aflatoxin content of the maize samples was found to be in the range 60–120 ng/g by the pressure mini-column method and 92 ng/g by HPLC. The naturally contaminated specimen used in the present trial was analysed 'blind' so as to minimize analyst bias. Analysis of sample I by the ten collaborators indicated a level of aflatoxin in the range 60–120 ng/g of maize, which corresponds with the value obtained at this Institute. However, in the case of sample II, only eight analysts reported 60–120 ng/g of aflatoxin. In case of sample III, all the analysts reported that the sample was negative for aflatoxin (table 1). The difference could be attributed to analyst error. The purpose of dividing the same contaminated specimen into samples I and II was to check whether there was any variation in analysis by individual workers. In the present trial, a specified methodology for sample preparation and aflatoxin extraction, and prepacked mini-columns were used, minimizing differences in technique between the individual analysts. From the results of the present collaborative trial, it can be concluded that there was a high degree of reproducibility and repeatability of the method. The pressure mini-column method can therefore be used as a rapid screening and detection method for aflatoxins.

The concept of collaborative studies for analysis of any food contaminant has been introduced in Public Health Laboratories for the first time in India. Such collaborative trials are routinely carried out in the USA[15]. The advantages of collaborative trials for specific contaminants include: (i) validation of method, (ii) minimizing inter-laboratory analytical errors, (iii) enhancing the accuracy and analytical capacity of analysing laboratories, and (iv) removing existing variations.

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ISOLATION AND CHARACTERIZATION OF SEVERAL SOIL PHAGES THAT LYSE *BACILLUS BREVIS*

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PHAGES of *Bacillus brevis* have been isolated and investigated for a variety of reasons, including the problems they cause in the food industry and their use as taxonomic tools in genetic studies[1-3]. Several workers have demonstrated the use of these phages in recombinant DNA technology[1-5]. Mainly two factors are vital for a phage to become a useful vector. First, the phage should have a wide host range, and second, it must also be possible to transfect the phage DNA or its recombinant molecules. In the present investigation several soil samples were screened for phages that could lyse *B. brevis*. I have described the molecular characterization of some of these phages.

For the isolation of phages soil samples (about 50 g) were incubated for 4–6 days in phage medium[6] containing the phage-sensitive *B. brevis* strain (10^8 cfu/ml) at 30°C for 24 h under shaking conditions. After the incubation the culture filtrate was plated[6] to check for the presence of phage. In all, five phages, viz. BS12, BS25, BS65, BS138 and BS158, were isolated. Purification of the phages was done as described and lysozyme activity was measured according to Verma and Siddiqui[7]. Temperate phages could be distinguished from virulent phages by the appearance of plaques on the lysogenic strain. These lysogenic bacteria were immune to superinfection by the same phage. Moreover, in a culture of a lysogenic strain, free phage particles would be detected, since lysogenic strains would spontaneously go into lytic cycle at a low level. Exposure to ultraviolet light increased the lytic response. Serological properties, host range, melting curves of phage DNAs and restriction analysis were done by following methods described elsewhere[8-10]. For transfection small amounts (5 to 10 μg) of phage DNA were mixed with 10^8 cells of *B. brevis* and 0.5 ml of 25% (w/v) polyethylene glycol in 10 mM calcium chloride at pH 8 was added. After 10 min of incubation at 30°C the contents were centrifuged briefly and plated on a lawn of the sensitive strain of *B. brevis*.

Ten soil samples were tested and five phages, viz. BS12, BS25, BS65, BS138 and BS158, were isolated. No real differences were observed in isolation efficiency between shaking cultures or standing cultures at 30°C. High phage titres (in the range of 10^{10} pfu/ml) were obtained from confluent plate lysates.

All the phage heads were of an icosahedral form, although phage head size and tail length varied considerably (table 1). Short tail fibrils (6 to 10) were attached to the base plate of each phage. Continuous cesium chloride gradients showed that the bouyant density of these phages varied between 1.5 and

Table 1 *Morphological characterization of B. brevis phages*

[illegible]	Phage dimensions (nm)				Plaque morphology		
	Head		Tail				
	Length	Width	Length	Width	Size (nm)	Transparency	Edge
[illegible]	140.8	130.0	350.5	15.0	1.5	Turbid	Diffuse
[illegible]	155.0	146.5	405.0	15.0	1.6	Clear	Sharp
[illegible]	132.8	130.5	350.5	14.5	1.4	Clear	Sharp
[illegible]	154.0	140.0	300.0	13.5	1.5	Clear	Sharp
[illegible]	140.0	150.0	410.0	14.0	1.5	Turbid	Diffuse

[illegible] virus was mixed with phage samples to serve as internal standard for phage [illegible]

[illegible] strains of *B. brevis*, 10 strains of [illegible] and three strains of *Escherichia coli* were [illegible] infection susceptibility to the five phages [illegible] in this work. Plaques were detected in [illegible] *B. brevis*; the other bacteria were [illegible] these phages. Phage BS138 could grow [illegible] *typhimurium* as well. Serologically all [illegible] were alike (neutralized by antisera of [illegible] in the present investigation), and they [illegible] neutralized by antisera to *B. subtilis*, [illegible] or *E. coli* phages.

[illegible] naturation experiments provided evidence of double helical secondary structure of the [illegible] DNAs. The melting profile of all phage DNAs [illegible] a narrow helix coil transition typical of double stranded DNA. The melting temperature [illegible] BS65 and BS158 was 64°C (G + C [illegible]) and for BS25 and BS138 it was 77°C ([illegible] mol%). The low melting temperature [illegible] BS65 and BS158 suggests the presence of [illegible] in the genome of these phages[11].

[illegible] is a significant enzyme produced by [illegible] cells in the later stages of infection. The [illegible] lysozyme production were measured (table 2) [illegible] was observed that the highest amount of [illegible] was produced by BS138-infected cells. This [illegible] because BS138 is a virulent phage.

[illegible] infection could be observed by plaque formation [illegible] regeneration plates after 24–30 h of regene- [illegible] by soaking regeneration plates with 4 ml [illegible] buffer and testing the latter suspension for the [illegible] of phage DNA[12]. For transfection experiments [illegible] DNA was used and the number of [illegible] centres was determined by counting the [illegible] of plaques on the transfectant plate. In [illegible] investigate whether the phage DNA was [illegible] in the chromosome or excised as an [illegible] element, I attempted to isolate the extrachromosomal element, if any, but the results were negative. This suggests that these phages probably integrate in the chromosome (the experiment was performed by cesium chloride gradient centrifugation as suggested by Joshi *et al.*[10])

Table 2 *Lysozyme activity in B. brevis infected with different phages*

Time after infection (min)	Lysozyme activity (units/ml) in cells infected with				
	BS12	BS25	BS65	BS138	BS158
0	0	0	0	0	0
5	0	0	0	0.5	0
10	0	0.3	0.4	2.0	0
15	0.1	0.5	0.6	3.5	0.4
20	0.2	0.8	0.8	5.0	0.8
25	0.8	3.0	2.0	12.0	2.0
30	2.0	5.0	5.0	16.0	5.0
40	5.0	8.0	7.2	18.0	5.0
50	8.7	8.0	7.5	18.5	5.0
60	8.8	8.0	7.5	18.5	5.5
70	8.8	8.0	7.5	18.5	5.5
80	8.8	8.0	7.5	18.5	5.5

The procedure for the assay of the enzyme has been described earlier[13].

Of the five phages isolated, four (BS12, BS25, BS65, BS158) were temperate and BS138 was a virulent phage. The temperate phages could switch to lytic cycle at a rate of about 2% of the number of viable cells. The transition from temperate to lytic can be enhanced by exposure of the lysogenic strain to ultraviolet radiation (240–280 nm), although long exposure decreases the number of viable cells (table 3).

The molecular weights of the phage DNAs, as determined by cesium sulphate gradient analysis, were as follows: BS12, 14.8 kb; BS25, 18.8 kb; BS65, 15.3 kb; BS138, 18.3 kb; BS158, 16.5 kb. The restriction maps of the phage DNAs are shown in figure 1. ^{32}P.

Table 3 *Effect of ultraviolet exposure on the viability of phages*

Time of exposure (min)	Per cent survivors remaining after exposure				
	BS12	BS25	BS65	BS138	BS158
No exposure	100	100	100	100	100
5	98	95	98	97	98
10	95	93	96	92	93
15	81	83	83	84	82
20	72	74	72	71	72
30	51	49	51	48	47
40	31	32	30	30	31
50	13	15	13	12	11
60	0.1	0.4	1	0.5	0.1

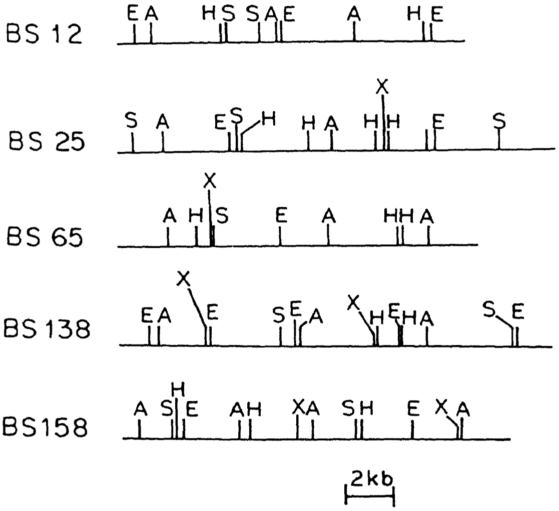

Figure 1. Restriction maps of *B. brevis* phages BS12, BS25, BS65, BS138 and BS158. E, *Eco*RI; A, *Ava*I; H, *Hin*dIII; S, *Sal*I; X, *Xho*I.

Labelling of phage DNAs using the Klenow fragment of DNA polymerase resulted in the labelling of only two *Eco*RII fragments (BS12 and BS138), two *Sal*I fragments (BS25), and two *Ava*I fragments (BS158 and BS65), all of which lie at the ends of the respective restriction maps (figure 1) indicating that the DNAs of these phages have 5′ protruding single-stranded ends. Further experimentation suggested that the single-stranded protrusions are complementary in base sequence but not palindromic.

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PLASMID-ENCODED PRODUCTION OF HYDROCARBON-SOLUBILIZING FACTOR BY *PSEUDOMONAS* PG–I

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ISOLATION of a polymeric hydrocarbon-solubilizing substance produced by *Pseudomonas* PG–I during growth on liquid and solid hydrocarbons has been reported previously[1, 2]. Production of extracellular alkane-solubilizing factor by the yeast *Endomycopsis lipolytica* YM has also been reported[3]. It was shown that hydrocarbon solubilization by solubilizing factors could fully account for substrate uptake and growth of the organisms on hydrocarbons.

Many reports have appeared on the specific action of sodium dodecyl sulphate (SDS) in the removal (curing) of bacterial plasmids[4–7]. This communication reports that synthesis of hydrocarbon-solubilizing factor in *Pseudomonas* PG–I is plasmid-dependent, as plasmid freed organism failed to utilize alkanes. This barrier could be overcome by the use of artificially emulsified substrate or exogenous addition of the solubilizing factor.

The characteristics and conditions for culture of *Pseudomonas* PG–I were described previously[1,2]. Hexadecane-emulsifying and solubilizing factor (PG–I $ESFC_{16}$) was isolated from cell-free broth obtained from a culture of *Pseudomonas* PG–I on *n*-hexadecane by precipitation with chilled acetone[1].

For plasmid curing by SDS, 50 ml of mineral medium was inoculated with a loopful of *Pseudomonas* PG–I culture, *n*-hexadecane was added as sole carbon source for 12 h and the culture shake-incubated. SDS was added to a final concentration of 800 μg/ml and growth allowed to continue for another 12 h. Serial dilution up to 10^{-7} was carried out and 0.1 ml was plated on mineral medium. For development of colonies, plates were incubated at 37°C in an atmosphere of liquid petroleum gas–air mixture (1:3). Colonies that did not grow on liquid *n*-hexadecane were carefully selected. *Pseudomonas* PG–I was also grown on 50 ml minimal medium containing 1 g sodium acetate. Five ml of the culture was retransferred to fresh medium and allowed to grow for a further period of 24 h. The isolation pattern was followed as before except that colonies were plated on nutrient agar medium.

For growth on liquid petroleum gas (LPG) agar–mineral medium slants were placed inside an evacuable gas-tight container containing LPG–air mixture (1:3) at 37°C for 2 days. For growth on hydrocarbon vapours, slants containing inoculum at the top were utilized. The liquid hydrocarbons (0.3 ml) were injected to the bottom of the test tubes using a syringe with a long needle. Tubes were incubated at 37°C for 2 days. The alkane solution at the bottom and the inoculum at the top of the slant were separated by about 3 inches in all the cases and growth occurred only by virtue of utilization of the alkane vapour.

The plasmid-cured strain failed to grow on liquid *n*-hexadecane presented in its natural physical form (figure 1A). But when the alkane was artificially emulsified and solubilized very good growth was observed, almost to the level of the native strain. As described previously[2], such artifically emulsified and solubilized hydrocarbon in shake-incubation could generate more solubilized hydrocarbon, in addition to the solubilized hydrocarbon already present.

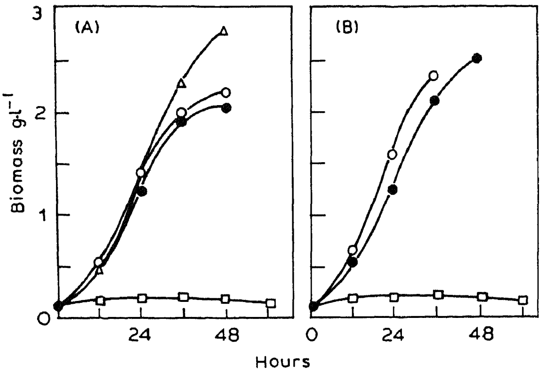

Figure 1. Growth of native and plasmid-cured *Pseudomonas* PG–I on various substrates. **A**, Native strain on liquid *n*-hexadecane (△), and plasmid-cured (SDS) strain on liquid *n*-hexadecane (□), liquid *n*-hexadecane + PG–I $ESFC_{16}$ (●), and emulsified *n*-hexadecane (○). **B**, *Pseudomonas* PG–I pregrown on sodium acetate, on liquid *n*-hexadecane (□), liquid *n*-hexadecane + PG–I $ESFC_{16}$ (●), and emulsified *n*-hexadecane (○).

As shown in figure 1 PG–I $ESFC_{16}$ (100 mg%) stimulated growth of the plasmid-cured strain on liquid *n*-hexadecane. The findings suggest that with the loss of plasmid(s), the organism also lost its ability to produce solubilizing factor and was thus unable to utilize the hydrocarbon. It also appears (figure 1B) that *Pseudomonas* PG–I lost plasmid(s) during growth on sodium acetate. The plasmid curing appears to be permanent as the strain could not readapt to grow on liquid *n*-hexadecane even after prolonged incubation for 10 days.

Although the plasmid-cured strain could not utilize liquid *n*-decane, *n*-dodecane and *n*-hexadecane, good growth was obtained when these alkanes were presented in the vapour phase in a closed solid culture system. Similarly the strain could grow very well on LPG.

The results, that plasmid-cured cells could utilize gaseous hydrocarbons and artificially emulsified and solubilized alkanes for growth, indicate that the enzymatic mechanism for utilization of solubilized hydrocarbon was intact in the plasmid-cured cells. The obvious implication is that the synthesis of these enzymes is not determined by plasmids in this organism. In the dimorphic yeast *Saccharomycopsis lipolytica* the genetic determinants for alkane-oxidizing enzymes were reported to be located on the chromosomes[8]. Chakraborty *et al.*[9] reported involvement of plasmids in degradation of hydrocarbons, including short-chain-length alkanes. Chakraborty[10] also documented plasmid-mediated degradation of simple aromatics.

1 August 1988; Revised 10 January 1989

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BIOSYNTHESIS OF PYRIMIDINES DURING GERMINATION OF BLACK GRAM SEEDS: CHANGES IN THE LEVELS OF PARTICIPANT ENZYMES

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DRAMATIC changes in rates of respiration, and synthesis and degradation of macromolecules, such as proteins, nucleic acids and starch, have often been observed in seeds during germination[1]. Nevertheless, little is known about the metabolism of pyrimidine nucleotides in germinating seeds. The present study was designed to determine the factors that affect the rate of biosynthesis of pyrimidine nucleotides during germination. We determined the levels of some key enzymes of the *de novo* and salvage pathways of biosynthesis of pyrimidine nucleotides, and followed changes in levels of 5-phosphoribosyl-1-pyrophosphate (PRPP) and ATP, donors of phosphoribosyl and phosphate groups, respectively, in the biosynthesis, in the cotyledons and embryonic axes of germinating black gram seeds.

Seeds of black gram (*Vigna mungo* L.) were sterilized and germinated on 0.55% agar gel in the dark at 27°C under aseptic conditions[2,3]. After a fixed length of time, cotyledons and embryonic axes were separated and analysed. Dry seeds were also used for the assays of enzymatic activities. Extraction of enzymes from seeds and seedlings was performed as described by Nobusawa and Ashihara[4]. The activities of enzymes were assayed radiochemically as described earlier[5-7]. For determination of levels of PRPP, samples (approximately 2 g fresh weight) were frozen rapidly with liquid N_2 and powdered with a chilled mortar and pestle. The powder was homogenized with 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM NaEDTA and 100 mM KF in a glass homogenizer. The homogenate was centrifuged at 23,000 *g* for 20 min at 2°C. The resultant supernatant was immediately used for assays. The level of PRPP was determined radiochemically[8]. For determination of ATP, samples (approximately 0.5 g fresh weight) were frozen in liquid N_2 and homogenized with 6% perchloric acid in a glass homogenizer. After centrifugation, the acid extracts were neutralized with KOH, and levels of ATP were determined with a Packard Pico-Lite luminometer, Model 6100, using firefly luciferase and luciferin[9].

Changes in the fresh and dry weights and in the levels of DNA and RNA in pairs of cotyledons and in individual embryonic axes of black gram seeds during germination are essentially identical, as we found in earlier studies[2,10] (data not shown).

The levels of selected enzymes involved in the biosynthesis of pyrimidine nucleotides *de novo*, i.e. carbamoyl-phosphate synthetase (CPSase), aspartate transcarbamoylase (ATCase), orotate phosphoribosyl transferase (OPRTase) and orotidine-5′-monophosphate decarboxylase (ODCase); the level of two enzymes involved in the pyrimidine salvage pathway, i.e. uridine kinase (UKase) and uracil phosphoribosyltransferase (UPRTase); and the level of PRPP synthetase were determined in extracts of dry seeds and of cotyledons and embryonic axes of seedlings at various stages of germination. Since it has been suggested that an enzyme complex of OPRTase and ODCase exists in higher plants[6], the combined activities of these two enzymes were measured. All of the enzymes listed were present in dry seeds at high levels (table 1). In cotyledons, levels of some of the enzymes, namely CPSase, ATCase and UKase,

Table 1 *Levels of enzymes involved in the biosynthesis of pyrimidine nucleotides in dry seeds (0 h) and in cotyledons and embryonic axes of black gram seedlings*

Enzyme		0 h	24 h	48 h	72 h	96 h
Carbamoyl-phosphate synthetase	Cotyledon	0.20	0.46	—	0.28	0.02
	Embryonic axis		0.03	0.24	0.50	0.60
Aspartate transcarbamoylase	Cotyledon	0.94	4.12	2.30	0.50	0.12
	Embryonic axis		0.30	0.72	1.14	3.86
Orotate phosphoribosyltransferase and orotidine-5′-monophosphate decarboxylase	Cotyledon	2.31	2.26	2.22	1.81	0.65
	Embryonic axis		0.38	0.62	0.94	1.58
Uridine kinase	Cotyledon	0.96	1.90	1.50	0.64	0.38
	Embryonic axis		0.10	0.22	0.26	1.98
Uracil phosphoribosyltransferase	Cotyledon	0.22	0.12	0.06	0.05	0.05
	Embryonic axis		0.06	0.08	0.12	0.38
Phosphoribosylpyrophosphate synthetase	Cotyledon	2.69	2.51	1.62	1.27	1.05
	Embryonic axis		0.42	0.93	1.05	3.60

Enzymatic activities are expressed as mU per seed, per individual pair of cotyledons, or per embryonic axis of the seedling. Whole seeds were used for the 0-h sample which consisted of dry seeds. The values are averages of results from duplicate samples. —, Not determined.

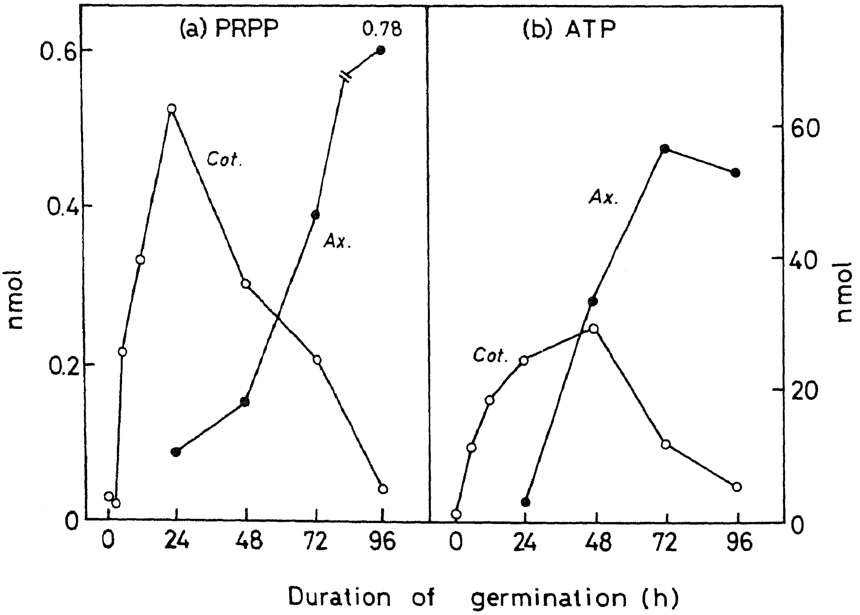

Figure 1. Changes in the levels of (**a**) 5-phosphoribosyl-1-pyrophosphate and (**b**) ATP in cotyledons and embryonic axes of black gram seedlings. The levels are expressed as nmol per individual pair of cotyledons (○) or nmol per embryonic axis (●). The values are averages of results from duplicate samples from a typical experiment.

increased transiently during the early phase of germination and then decreased, but the levels of OPRTase–ODCase, UPRTase, and PRPP synthetase all fell after initiation of germination. In embryonic axes, the levels of all the listed enzymes related to the biosynthesis of pyrimidine nucleotides increased with the growth and development of the embryos (table 1). The presence of CPSase, OPRTase and ODCase has also been noted in dry seeds of pea plants[11,12].

We determined the levels of PRPP and ATP in various tissues. The levels of these compounds were extremely low in dry seeds, but they increased rapidly in cotyledons after imbibition, reached maximum levels at 24 h (PRPP) and 48 h (ATP), and decreased subsequently (figure 1). A rapid increase in the level of PRPP has also been observed in the cotyledons of peas, but the maximum was achieved 12 h after the start of imbibition[11]. The levels of PRPP and ATP in the embryonic axes increased with growth, but the patterns of incremental changes in the levels of these two compounds were slightly different (figure 1). An increase in ATP levels during the first hours of imbibition has been observed in seeds of several plants[1], although conflicting results have been reported about the reactions involved in the formation of ATP during the very early stages of germination[14-16]. An increased level of ATP in cotyledons of black gram seeds seems to act as a trigger to set off the subsequent formation of phosphorylated high-energy compounds, such as PRPP, with a possible resultant enhancement in the rate of biosynthesis of pyrimidine nucleotides.

The results obtained here and those of previous studies using radioactive tracers and an inhibitor of protein synthesis[2,3] lead to the following conclusions. (i) The enzymes that participate in the biosynthesis of pyrimidine nucleotides are present at adequate levels but are inactive because of dehydration in cotyledons of dry seeds and become functional after hydration of the enzyme proteins with imbibition. (ii) The levels of ATP and PRPP increase with imbibition by the seeds and enhance the activity of enzymes involved in the biosynthesis of pyrimidine nucleotides. (iii) At a later stage of germination, constituents of cotyledons are hydrolysed by the degradative enzymes, which are synthesized during germination, and the resultant products, such as amino acids, nucleosides, nucleobases and sugars, are transported to the embryonic axes and utilized for the active biosynthesis of pyrimidine nucleotides. (iv) In the embryonic axis, most of the enzymes involved in the synthesis of pyrimidine nucleotides, as well as ATP and PRPP, are synthesized *de novo*.

19 January 1989

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STABILITY OF INDOMETHACIN TO CONDITIONS OF ENZYMATIC RELEASE FROM TISSUE—SOME INTERESTING ASPECTS

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WE have earlier[1,2] proposed new methodologies for enzymatic hydrolysis of tissues using the two enzymes neutral proteinase (from *Bacillus subtilis*) and papain (from papaya). It was then of interest to investigate the stability of indomethacin (an analgesic) to the conditions employed for enzymatic hydrolysis of tissues using these two enzymes. Aqueous solutions of the drug were incubated with (i) buffer alone, and (ii) buffer and enzyme, prior to extraction and quantitation of indomethacin in the reaction mixture by HPLC. The results proved to be interesting. The concentration of indomethacin in the reaction mixture after enzymatic action by neutral proteinase and subtilisin A revealed that only 67% and 38% respectively of the drug originally present could be recovered. In contrast, incubation with papain showed no degradation of indomethacin and complete recovery (100%) was possible. To test for benzoic acid and its derivatives as possible degradation products the chromatographic method of Fewster and Hall[3] was employed. The results were however negative, pointing out that indomethacin is transformed by neutral proteinase and subtilisin A by an as yet unknown mechanism.

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ANNOUNCEMENTS

ALL INDIA COURSE ON VACUUM SCIENCE AND TECHNOLOGY

The course, organized by the Indian Vacuum Society in collaboration with the Centre for Advanced Technology, Indore, will be held at the Centre for Advanced Technology, Rajendranagar, Indore, during October 16–26, 1989. For application forms and details contact: Shri A. S. Raja Rao, Convener, All India Course on Vacuum Science and Technology, Centre for Advanced Technology, P.O. Rajendranagar, Indore 452 012, India.

Completed forms must reach the Convener before 15 September 1989.

FIRST NATIONAL SEMINAR ON CLINICAL NUTRITION IN VASCULAR MEDICINE

The seminar will be held at M.L. Theatre, KEM Hospital, Bombay, on 17 September 1989. Deadline for registration 2-9-1989. For details please contact: Dr M. E. Yeolekar, Professor of Medicine, Secretary General, National Seminar on Clinical Nutrition, K.E.M. Hospital, Parel, Bombay 400 012, India.

NATIONAL SEMINAR ON THE ROLE OF SOIL AND WATER CONSERVATION IN MODERN AGRICULTURE

The seminar will be held at the Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, during October or November 1989. For details contact: Dr G. B. Roy, Organizing Secretary, National Seminar on SWC, Department of Agricultural Energy, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741 252, India.

BOOK REVIEW

Annual Review of Computer Science, vol. 2, 1987, pp. 565, (ed.) J. F. Traub, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, CA 94306, USA), Price: US$ 39, elsewhere $ 42.

The second volume of *Annual Review of Computer Science* is a collection of good papers providing up-to-date information in the most important areas of research in computer science, including artificial intelligence (AI). The papers provide enough background material so that they can be read, understood and appreciated independently. They also provide a good list of references for further information.

Lisp evolved to serve the world of evolutionary, extrapolatory programming. A modern Lisp system contains a lot of built-in facilities compared to other languages. It is this extensive base that separates a professional Lisp implementation from a toy implementation. The paper 'Common Lisp' (S. E. Pahlman) explains the need for common, stable Lisp, facilities offered by a typical Lisp environment, its role in evolutionary programming, and hardware essential for such an environment.

Designing, understanding and reasoning about distributed systems are complicated because of the uncertainties inherent in the system and lack of global knowledge. The paper 'Using reasoning about knowledge to analyse distributed systems' (J. Haplern) describes how to formally use knowledge in distributed systems and the gains of such formalisms. The systems could be computer systems, robots or people. Common knowledge is a prerequisite in the co-ordinated actions and agreement, and this seems to be a useful tool for specifying, synthesizing and verifying protocols.

The discovery of various constraints and techniques for using them are essential to guide inferences from 2-D image data to 3-D scene descriptions. The paper The emerging paradigm of computational vision' (S. Zucker) describes computational vision along with the related issues of biological and engineering vision systems. General computational theories hold abstractly for all applications and provide the means for integrating the diverse constraints.

The intelligent robot must be able to reason symbolically. The mobile robot is always encountering new and unexpected events. So static models or preloaded maps are inadequate to represent the robot world. The paper 'Vision and navigation for the Carnegie–Mellon Navlab' (C. Thorpe *et al.*) describes the tools a mobile robot (Carnegie–Mellon Navlab) uses to bridge the chasm between the external world and its internal representation, such as sensors, image understanding to interpret sensed data, geometrical reasoning, concepts of time and of motion over time.

An intelligent artefact must be capable of reasoning about the world it inhabits. The technical problem of AI is to characterize the pattern of reasoning required of such an intelligent artefact. One such reasoning technique is nonmonotonic reasoning. The paper 'Nonmonotonic reasoning' (R. Reiter) describes various nonmonotonic abstractions such as default logic. Circumscription along with some objections such as probability theory may be more appropriate. Nonmonotonicity appears to be the rule rather than the exception in much of what passes for human common-sense reasoning.

Logic and deduction is related to problem solving in more than one way. The paper 'Logic, problem solving and deduction' (D. V. McDermott) describes the work on such relationships. The role of logic in AI depends on several issues such as how effective the automated deduction algorithm can become, how many problems can be cast as deductive problems, and how important logic is to the study of knowledge representation.

Reasoning about actions and plans is fundamental to the development of intelligent machines that are capable of dealing effectively with real-world problems and is one of the areas of investigation in AI research. The paper 'Planning' (M. P. Georgeff) discusses the issues that are relevant in reasoning about actions and plans and describes how to represent actions and events, and plan synthesis.

Language generation and explanation systems allow computer systems to respond to their users in natural language. Language generation can aim to produce short answers, paragraph-length responses or summaries. Explanation is a subtype of language generation. The paper 'Language generation and explanation' (K. R. McKeown and W. Swartout) describes the problem of language generation, the range of system organizations and solutions used, and applications for which generation systems have been built.

Search is a universal problem-solving mechanism in AI. Typical search tasks range from solving Rubik's cube to playing games such as chess, to solving complex practical problems such as medical diagnosis. The paper 'Search techniques' (J. Pearl and R. E. Korf) discusses various search techniques such as brute-force techniques, heuristic techniques and game-tree searching techniques.

Software development requires knowledge about software engineering and programming techniques, the application domain, the machine upon which the software will be run, and the decisions already made about the implementation of the software under development. The current software tools are adept at dealing with the clerical details of programming and are of little support in knowledge-intensive aspects of software development. The paper 'Knowledge-based software tools' (D. R. Barstow) describes the various research efforts in the development of software tools that can exploit the knowledge to some extent like a programmer. These tools can assist the programmer in the development of systems and programs and may offer substantial productivity gains.

Protocols provide communication between local as well as remote processes. Protocols can also be used to integrate a partitioned complex function on a single processor system. There is a need for standard protocols so that teleprocessing-equipment manufacturers will be able to interconnect various equipment efficiently. The paper 'Network protocols and tools to help produce them' (H. Rudin) describes the formal protocol specification concepts and tools that may be driven from a machine-readable formal specification.

Temporal logic can be used for specifying and verifying concurrent programs and for verifying network protocols. The construction of proof in hand is very tedious. The paper 'Research on automatic verification of finite-state concurrent systems' (E. M. Clarke and O. Grumberg) describes the syntax and semantics of temporal logic and its application in verifying concurrent systems.

Many computational problems have benefitted greatly from the study of the mathematical structure underlying the problems and this may help in devising more efficient algorithms than naive enumeration. So it may be fruitful to study the mathematical structure underlying the NP-hard problems and develop new techniques for solving them. The paper 'Algorithmic geometry of numbers' (R. Kannan) provides a self-contained introduction to algorithmic geometry of numbers and surveys its application to cryptography and diophantine approximations among others.

Computational algebra is helpful in the construction of computer systems that enable scientific and engineering users to carry out mathematical manipulations automatically. The paper 'Computer algebra algorithms' (E. Kaltofen) describes the development of efficient arithmetical algorithms and the relationship of computer algebra to other fields such as logic programming and AI.

Linear programming has enormous practical importance. Improvements by orders of magnitude in the efficiency of methods would open up many new application areas, with substantial economic gains. The paper 'Linear programming 1986' (N. Megiddo) describes various algorithms such as Karmarkar's algorithm and linear rescaling algorithm, and surveys recent algorithms for linear programming.

The life-threatening consequences and significant economic impact of computer failure are ponderous. System reliability is the major concern of manufacturers, semiconductor fabricators and software engineers. One of the principal mechanisms for achieving high reliability and high availability in digital systems is fault tolerance. The paper 'Techniques and architectures for fault-tolerant computing' (R. A. Maxion *et al.*) provides a selected review of fault-tolerant computing.

Computers are transforming the way we learn and the way we work. Computers are being used for special education, adult literacy programmes and training in industry and the military. The paper 'Computers in education: a historical overview' (D. M. Kurland and I. C. Kurland) describes the development of educational computer applications and their impact on education at the pre-college level.

In a nutshell, the volume has something for everyone presented in concise fashion.

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NEWS

More facilities for university research

University research in India will get a boost from access to major facilities of the Department of Atomic Energy (DAE) when the proposed Inter-University Consortium for DAE Facilities is set up. A memorandum of understanding for the setting up of the Consortium has been signed by Prof. Yash Pal, Chairman, University Grants Commission (UGC), and Dr M. R. Srinivasan, Chairman, Atomic Energy Commission.

The Consortium will allow greater interaction between university students and teachers and scientists at DAE's institutions. DAE's research facilities, particularly the Dhruva reactor at Bombay, the Variable Energy Cyclotron Centre at Calcutta, and the Synchrotron Radiation Source Centre at Indore, will be accessible to university researchers.

The UGC has earlier established the Nuclear Science Centre, an inter-university centre for research in nuclear sciences, at the Jawaharlal Nehru University campus, New Delhi, and the Inter-University Centre for Astronomy and Astrophysics, Pune. There is also a proposal for an inter-university centre for high-energy physics at Delhi, Jammu or Chandigarh.

The setting up of the Consortium and the centres is in line with the recommendation, in the National Policy on Education 1988, of autonomous national research facilities within the university system.

Restriction enzymes to be made in India

India's first venture capital biotechnology company that will make enzymes for genetic engineering has been launched. The new company, Bangalore Genei Private Ltd, Bangalore, will make restriction enzymes. It has been set up by Dr P. S. Babu, a theoretical physicist-turned-molecular biologist, and Dr K. Prasad, an immunologist, both of whom have been associated with the Indian Institute of Science (IISc), Bangalore. A large part of the funding for the company is by the Technology Development and Information Company of India (TDICI).

The new company will obtain the know-how for the manufacture of the enzymes from Astra Research Centre, Bangalore, which does research in medical biotechnology and is developing diagnostic kits for a variety of tropical diseases. The Centre for Genetic Engineering, IISc, will also assist the new company.

Restriction enzymes are currently being imported by individual user laboratories and in bulk quantities by the CSIR Centre for Biochemicals, New Delhi. The new company will initially make the more important and commonly used restriction enzymes.

Twenty-five years of radio astronomy at TIFR

The radio astronomy group of the Tata Institute of Fundamental Research, Bombay, completed twenty-five years of work in April this year. The event was celebrated at Udhagamandalam (Ooty) on 20 April. A two-day international symposium on 'The large-scale structure and evolution of the universe' and an open house for students and general public at the radio astronomy centre were also held.

The celebration was inaugurated by R. Venkataraman, President of India. Prof. Virendra Singh, Director of TIFR, recalled that R. Venkataraman, as Minister of Industries of the Government of Madras, had helped acquire the land in Ooty for setting up the centre. Dr M. R. Srinivasan, Chairman, Atomic Energy Commission, Prof. M. G. K. Menon, Scientific Adviser to the Prime Minister, and Prof. Govind Swarup, Director, Giant Metre-wave Radio Telescope Project of the TIFR, recalled the contributions of Meghnad Saha, S. Chandrasekhar, Homi Bhabha, D. S. Kothari and M. K. V. Bappu.

The facility at Ooty includes a metre-wave radio telescope. The TIFR group, headed by Prof. Govind Swarup, has made significant contributions. The Ooty radio telescope has provided accurate positions, sizes and flux densities of more than a thousand radio sources. The group has also discovered a giant radio galaxy in the southern hemisphere of the sky. TIFR has plans to build the world's biggest radio telescope at Narayangaon near Pune by 1993.

ANNOUNCEMENTS

PROF. J. V. BHAT–EUREKA FORBES AWARD FOR RESEARCH IN MICROBIOLOGY

The award (Rs. 10,000) is for excellence in research in microbiology conducted in a laboratory in India over the last 5 years (1985–89).

For details and application/nomination forms write to: The Prof. J. V. Bhat Memorial Committee, c/o Dr Yvonne Freitas, P.O. Box 80, G.P.O., Bombay 400 001.

Entries must be received by 15 September 1989.

PROF. T. R. GOVINDACHARI 60TH BIRTHDAY COMMEMORATION AWARD IN ORGANIC CHEMISTRY

The award (Rs. 5000) is to be made to a distinguished Indian organic chemist working in India with a minimum of 15 years' teaching and research experience.

Applications/nominations containing bio-data (4 copies), a five-page summary of research accomplishments, a list of publications, and one set of reprints must reach The Registrar, University of Madras, Madras 600 005, by 14 September 1989. For conditions of award send a self-addressed and stamped (Rs. 1.50) envelope.

MEETING REPORT

IAU/URSI SYMPOSIUM ON RADIO ASTRONOMICAL SEEING

Signals from astronomical sources are in general modified as they travel through the intervening medium between the source and the observer. The degree of modification due to different regions of the intervening medium varies with frequency. In the radio frequency range, the effects of the medium have been observed for quite some time now and have been used to study the properties of the medium. Knowledge of these properties helps greatly in evolving fruitful strategies for astronomical observation and in recovering information on celestial sources that would have been available in the absence of the medium. Although a lot of observational and theoretical work has been going on in this field for a long time, this symposium, held in Beijing from 15 to 19 May 1989, was the first on 'radio astronomical seeing'. Various aspects of the intervening medium and its effect on radio astronomical observations were discussed.

At sub-millimetre and millimetre wavelengths, propagation effects in the troposphere are of serious concern. It is seen that humidity and temperature have large gradients as well as strong fluctuation with height owing to turbulence in this medium. The measured phase fluctuations resulting from the passage of signals through the medium show that the spectrum of irregularities is quite close to the Kolmogorov spectrum. The RMS phase fluctuations observed are found to be large in the daytime and during summer. From interferometric observations it is also seen that these phase fluctuations increase with observing frequency and the baseline used, and decrease with elevation. Single-dish measurements of refraction on radio sources at millimetre wavelengths indicate position wander of as much as 30" even over half-a-minute time-scales. The strength of refraction correlates well with the height of the inversion layer. The sky noise fluctuations contribute increasing correlated noise at shorter baselines while the RMS phase fluctuations are reduced. The sensitivity of observation with short baselines in this frequency range is thus affected.

The attenuation and wet delay caused by the troposphere are measured by using a water vapour radiometer working in the microwave band. Opacity of the medium can be estimated from meteorological data as well as from radio measurements. Good mean correlation observed in radiosonde data and data from radio measurements suggests that the radiosonde data can be used confidently for site evaluation.

The dynamic range of maps and resolution obtained is a strong function of season but atmospheric seeing can also vary on time-scales of days in 'good' weather conditions.

The contribution of the ionosphere to astronomical seeing was also discussed. This region of the intervening medium, extending from about 50 to 1000 km from the Earth's surface, assumes great importance in the metre and decametre wavelength range. The typical scale sizes of electron density irregularities in the ionosphere range from tens of kilometres to a few metres. These disturbances travel from the polar region to the equatorial region. Doppler-shift monitoring of these irregularities shows acceleration over times shorter than an hour. The seeing is affected most during post-sunset times. The critical frequency of the upper ionosphere (F2 layer), monitored using vertical sounding data, shows a very clear abnormal pre-dawn (03–05 LT) peak. The occurrence probability of such pre-dawn peaks is seen to decrease with increase in solar activity (sunspot number).

Ionospheric refraction measurements of signals from satellites indicate that the amount of refraction is directly proportional to the intensity of the travelling ionospheric disturbances (TID). Angular modulation caused by even moderate-intensity TID, with typical wavelength of ~100 km, can sometimes be larger by factors of 2–5 than the magnitude of refraction in the regular ionosphere. From metre-wavelength observations of astronomical sources it is seen that interferometric phase can have RMS fluctuations of ~1–2 radians. Faster phase changes have also been observed with increasing solar activity. Even during the minimum solar activity period, it is found necessary to correct for ionospheric refraction about every 30 seconds.

The horizontal distribution of the total electron content (TEC), which characterizes the phase changes, refraction and delays introduced by the ionosphere, can be deduced by using measurements of differential delay or Faraday rotation of satellite signals at two frequencies. When the sizes of ionospheric irregularities are much larger than interferometer baselines it is shown that the horizontal gradient in TEC of the ionosphere can be extracted from observations on strong radio sources, using statistical methods. In the presence of Faraday rotation in the ionosphere, it is also seen that, essentially, the visibility amplitude alone is modified.

While mapping large fields of view at metre and decametre wavelengths, the effects of the ionosphere need to be solved for. The number of isoplanatic patches, which decides the number of unknowns introduced owing to modification of the wavefront in the ionosphere, increases as the fourth power of the wavelength. However, many of the isoplanatic patches may be common in fields viewed by different antenna elements of telescopes such as the Very Large Array (VLA) and the Giant Metre-wave Radio Telescope (GMRT). This possibility can effectively reduce the number of unknowns due to the ionosphere. Image reconstruction techniques such as self-calibration using constraints like closure phase and closure amplitude can be supplemented with some additional constraints by making multifrequency observations. It is also suggested that additional information could be obtained by effectively defocusing the dish beam in different ways to get different linear combinations of contributions from different isoplanatic patches. At decametre wavelengths, it is suggested that one should plan for measurements of all required baselines simultaneously with a large number of small-diameter antennas. The subarrays should be used to determine the distortion introduced by the ionosphere and correct for it. Data corrupted by scintillations should not, in general, be used for map-making. It is not very clear whether ionospheric seeing is a strong function of geomagnetic latitude. Nevertheless, the GMRT proposed to be built near Pune, India, would be perhaps the first radio telescope for which the site has been selected on the basis of ionospheric seeing conditions.

Imaging in the presence of scintillation has different regimes depending on the strength of scattering and integration time. In the case of weak scattering a short integration regime corresponds to 'speckle' imaging while the long integration regime corresponds to the mean seeing disk. In the case of strong scattering there are regimes that correspond to (successively) the 'snapshot' image, 'average' image and 'ensemble-average' image as the integration time increases. Although scattering has been studied intensively by radio astronomers, there has been little investigation of the 'speckle' phenomenon found in optical scattering. Using high temporal and angular resolution data from VLA, the existence of speckles in interplanetary scintillation (IPS) scattering disks has been demonstrated. Such speckles lead to a number of conceptual and practical difficulties for correction of radio-astronomical seeing.

Investigation of the effect of solar wind on metre-wavelength VLBI (Very Long Baseline Interferometry) shows that coherence time reduces with decreasing source elongation from the Sun. Thus it may be required to choose suitably large elongations if large integration times are to be used. A similar conclusion is also reached when IPS limits on very-low-frequency VLBI are considered.

On the other hand, IPS has been extensively used to estimate angular sizes of compact sources. Routine monitoring of IPS shows that occasional enhancements in the scintillation index drift in elongation. When such an enhancement reaches 90° elongation, it correlates with a geomagnetic storm.

From VLA measurements of HI absorption distribution across a strong radio source (Cas-A), it is shown that neutral hydrogen in interstellar medium (ISM) may have fine structures down to scales of 0.05 pc. An interesting observation of a possible extragalactic source was reported, where the source image was considerably enlarged by ionized gas in the intervening galactic HII region. The random changes in the position of compact sources that result from refractive effects are observed to be much smaller than the apparent image size scatter broadened by diffractive-scale irregularities in the ISM. It is shown that the image sizes observed are consistent with the predictions of the Kolmogorov theory of turbulence applied to interstellar plasma. The small value of the position wander however may be due to observing over short times compared to the typical refractive time-scales. In the case of slow-intensity variations of pulsars, it is shown that observing over periods that are not much longer than the refractive time-scales would lead to gross underestimation of the true modulation index.

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ARTICLES

METAL COMPLEXES OF 6-METHOXY-2-BENZOTHIAZOLAMINE

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ABSTRACT

Complexes of Co(II), Cu(II), Zn(II), Cd(II) and Hg(II) halides with 6-methoxy-2-benzothiazolamine (L) [ML_2X_2 (M = Co, Cu, Zn; X = Cl, Br; and M = Cd, Hg; X = Cl, Br, I)] were isolated and characterized by electrical conductivity, magnetic susceptibility, reflectance and infrared spectral data. Electronic spectra indicate a tetrahedral geometry for the Co(II) complexes and distorted octahedral structure for the Cu(II) complexes. The ligand is proposed to be coordinated through the ring nitrogen in all these complexes, except the mercury complexes, where linkage through the amino nitrogen is indicated.

INTRODUCTION

SUBSTITUTED benzothiazoles are biologically active compounds[1,2]. It has been observed that metal ion complexation improves their biological activities[2-6]. Benzothiazoles are also interesting ambidentate ligands. 2-Benzothiazolamine and its methyl and halogeno derivatives have been studied for their ligand characteristics[7-13] but alkoxy-substituted derivatives have been little studied. We have initiated a study of the ligand behaviour of alkoxy derivatives of 2-benzothiazolamine[14] and this paper deals with the coordination compounds of Co(II), Cu(II), Zn(II), Cd(II) and Hg(II) with 6-methoxy-2-benzothiazolamine (L).

EXPERIMENTAL

Materials

6-Methoxy-2-benzothiazolamine (Koch–Light, pure grade) was used without further purification. Cobalt(II) chloride, cobalt(II) bromide and copper(II) chloride of BDH(LR); copper(II) bromide of Cieche, USSR; zinc(II) chloride of Loba(AR); cadmium(II) chloride of S.M.(LR); and cadmium(II) iodide and mercury(II) chloride of Glaxo Lab(AR) were used as such.

Preparation of the complexes

The complexes were prepared by mixing the respective metal salts and the ligand in 1:2 molar ratio in methanol, ethanol or acetone. However, ZnL_2Br_2, CdL_2Br_2 and HgL_2Br_2 were isolated by adding 2 mmol of KBr to preformed solution of the chloro complex obtained from the metal chloride and ligand. For HgL_2I_2, $HgCl_2$ solution in acetone was treated with NaI and filtered to remove the precipitated NaCl, and the filtrate was then treated with the ligand.

Analysis of the complexes

C, H and N were microanalysed at the Central Drug Research Institute, Lucknow, and Regional Sophisticated Instrumentation Centre, Chandigarh. Sulphur and halogens were estimated as usual[15]. The metals were estimated volumetrically using EDTA[15].

Physical measurements

IR spectra (caesium iodide discs) were recorded on a Perkin-Elmer 577 spectrophotometer in the 200–4000 cm^{-1} region. Molar conductivities were determined with a Philips conductivity meter, model PR 9500/90. Magnetic measurements were performed on a Gouy balance. Reflectance and nujol mull spectra were recorded in the 25000–5000 cm^{-1} (400–2000 nm) region on a Hitachi 330 UV-VIS-NIR spectrophotometer (RSIC, Chandigarh) against a $BaSO_4$ disc. Electronic spectra in solution were recorded on a Pye-Unicam PU 8600 spectrophotometer in the 50000–11111 cm^{-1} (200–900 nm) region.

RESULTS AND DISCUSSION

The compounds synthesized, and the analytical, physical, spectral and IR data are given in tables 1–3. The analytical data indicate the composition of the complexes to be ML_2X_2. The molar conductivity

Table 1 *Analytical data, colour, molar conductivity and magnetic moment of 6-methoxy-2-benzothiazolamine (L) complexes with Co(II), Cu(II), Zn(II), Cd(II) and Hg(II)*

Complex	Colour	Conductivity ohm^{-1} cm^2 mole^{-1}	Magnetic moment (BM)	% Found (% Calculated)					
				C	H	N	S	X	M
CoL_2Cl_2	Blue	6.8	4.8	39.0 (39.1)	3.2 (3.2)	11.2 (11.4)	12.9 (13.0)	14.6 (14.4)	12.1 (12.0)
CoL_2Br_2	Blue	9.3	4.4	33.5 (33.1)	2.6 (2.7)	8.9 (9.6)	10.8 (11.0)	2..4 (27.6)	10.1 (10.1)
CuL_2Cl_2	Black	46.0	1.9	39.6 (38.8)	3.4 (3.2)	11.0 (11.3)	12.1 (12.9)	13.3 (14.3)	12.5 (12.8)
CuL_2Br_2	Black	45.1	1.9	32.4 (32.9)	2.7 (2.7)	8.7 (9.5)	10.2 (10.9)	27.1 (27.4)	10.8 (10.8)
ZnL_2Cl_2	White	16.9	—	39.4 (38.7)	3.4 (3.2)	11.2 (11.3)	12.4 (12.9)	13.8 (14.3)	13.1 (13.1)
ZnL_2Br_2	Grey	20.3	—	32.1 (32.8)	2.9 (2.7)	9.9 (9.5)	10.5 (10.9)	27.2 (27.3)	11.3 (11.1)
CdL_2Cl_2	Grey	48.8	—	35.0 (35.3)	2.9 (2.9)	9.2 (10.3)	10.3 (11.7)	13.7 (13.0)	20.7 (21.6)
CdL_2Br_2	Grey	42.2	—	32.3 (30.3)	2.7 (2.5)	8.0 (8.8)	9.6 (10.1)	25.0 (25.3)	17.4 (17.7)
CdL_2I_2	White	50.0	—	27.2 (26.4)	2.3 (2.2)	6.5 (7.7)	8.7 (8.8)	— (34.9)	15.4 (15.4)
HgL_2Cl_2	White	23.3	—	30.8 (30.4)	2.6 (2.5)	8.1 (8.8)	9.9 (10.1)	11.1 (11.2)	31.5 (31.7)
HgL_2Br_2	Grey	23.3	—	27.7 (26.6)	2.3 (2.2)	7.0 (7.7)	8.1 (8.8)	22.0 (22.2)	28.0 (27.8)
HgL_2I_2	White	25.4	—	24.4 (23.5)	1.9 (1.9)	6.9 (6.8)	8.5 (7.8)	— (31.1)	24.7 (24.6)

values of all the complexes indicate their non-electrolytic behaviour in acetone or DMF. The magnetic moment of the cobalt chloro complex (4.74 BM) is on the borderline between the values for the tetrahedral (4.4–4.7 BM) and the octahedral (4.7–5.2 BM) structure, but the magnetic moment of the bromo complex (4.6 BM) indicates its tetrahedral geometry. However, the electronic spectra of these complexes definitely suggest that they are tetrahedral. For the Cu(II) complexes, the magnetic moments correspond to the normal one-electron case. The electronic spectra suggest that the Cu(II) complexes have a distorted octahedral approximating to square planar configuration.

ELECTRONIC SPECTRA

Cobalt complexes

The electronic spectra of the complexes were measured in acetone and the reflectance spectra in the form of $BaSO_4$ disc. The reflectance spectra of the complexes exhibit bands around the 16000 cm^{-1} region. The solution spectra also show bands in the 15873–15822 cm^{-1} region. The presence of bands in the same region both in the solid state and in solution indicates that there is no change in the coordination number or stereochemistry due to solvation. The order of $\Sigma m (\approx 3 \times 10^2)$ appears to suggest a tetrahedral geometry. The tetrahedral complexes of Co(II) are expected to show three bands corresponding to ν_1, $^4T_2(F) \leftarrow {}^4A_2(F)$; ν_2, $^4T_1(F) \leftarrow {}^4A_2(F)$; and ν_3, $^4T_1(P) \leftarrow {}^4A_2(F)$, but generally two bands for ν_2 and ν_3 corresponding to $^4T_1(F) \leftarrow {}^4A_2(F)$ and $^4T_1(P) \leftarrow {}^4A_2(F)$ are observed. The ν_1 band corresponding to $^4T_2(F) \leftarrow {}^4A_2(F)$ is expected to be in the 3000–5000 cm^{-1} region. Duff *et al.*[16] studied the magnetic moments and electronic spectra of halogeno complexes of cobalt with 2-thiazolamine and noted that the lower energy transition corresponding to ν_2 is split into three components, as expected for C_{2v} symmetry. We also

Table 2 *Electronic spectral data for some 6-methoxy-2-benzothiazolamine (L) complexes*

Complex (phase)		Observed (cm^{-1})			Calculated[21]					
		ν_2	ν_3	10 Dq	ν_2	ν_3	15B′	$\beta = \frac{15B'}{15B}$	B%	ν_2/ν_1
CoL_2Cl_2 (solid)		6250, 7353, 8696	15873, 17094 sh	4490	7695	16485	10710	0.67	32.4	1.7
CoL_2Cl_2 (solid)		5882, 6849, 8928	15504, 16666 sh	4440	7600	16086	10365	0.65	34.5	1.7
(acetone)	15873 (Σm 274)									
CoL_2Br_2 (solid)	—	5714, 6803, 8734	15384, 16666 sh	4335	7429	16025	10455	0.66	33.9	1.7
(acetone)	15822 (Σm 288)									
CuL_2Cl_2 (nujol mull)	32786, 20000 br									
(acetone)	28328 (Σm 2923), 20408 br (Σm 680)									
CuL_2Br_2 (nujol mull)	32786, 20000 sh									
(methanol)	27027 (Σm 1418), 20000 sh									

L′ = 2-Thiazolamine
15B for free ion = 15840 cm^{-1}.

observed a prominent band at 15503 cm^{-1} (sh 16666 cm^{-1}) and at 15384 cm^{-1} (sh 16666 cm^{-1}) in the cobalt chloro and bromo complexes respectively, which may be assigned to ν_3. The bands corresponding to ν_2 transitions are observed at 8928, 6849 and 5882 cm^{-1} in the chloro and at 8733, 6802 and 5714 cm^{-1} in the bromo complexes. The similarity in the reflectance spectra of the 6-methoxy-2-benzothiazolamine complexes and 2-thiazolamine complexes of Co(II) indicates similar geometry for the two series. In dichloro-bis(2-thiazolamine) Co(II), the metal atom is shown by X-ray studies to be tetrahedrally coordinated through the ring nitrogen atom[17]. The calculated values of β for the 2-thiazolamine and 6-methoxy-2-benzothiazolamine complexes are very close, which also indicates similar mode of linkage through the ring nitrogen atom in both cases.

Copper complexes

It is not possible to unambiguously assign the stereochemistry of Cu(II) complexes only on the basis of electronic spectral data in a limited range[18, 19]. The electronic spectra of the chloro complex in acetone and the bromo complex in methanol show a band around 28328 cm^{-1} (Σm, 2923) and 27027 cm^{-1} (Σm, 1418) respectively, which can be assigned as charge-transfer transitions[18]. A broad band around 20408 cm^{-1} (Σm, 680) in the chloro complex and a shoulder around 20000 cm^{-1} in the bromo complex suggest a probable square planar configuration[20]. However, as Cu(II) complexes frequently exist in a highly distorted stereochemistry[19], and the spectra of the solid complexes in nujol mull also show a broad plateau around 20000 cm^{-1} (along with the charge-transfer band at 32786 cm^{-1}), it appears more reasonable to assign a distorted octahedral stereochemistry for these mixed ligand complexes with CuN_2X_2 chromophore.

INFRARED SPECTRA

6-Methoxy-2-benzothiazolamine shows two bands

Table 3 *Principal IR bands (cm^{-1}) of 6-methoxy-2-benzothiazolamine (L) and its complexes*

Band assignment	L	CoL_2Cl_2	CoL_2Br_2	CuL_2Cl_2	CuL_2Br_2	ZnL_2Cl_2
$\nu_{NH_2(asym)}$	3365 s, sp	3420, 3340 s, sp	3410, 3330 s, sp	3365 s, msp	3385 s, msp	3400, 3330 s, mbr
$\nu_{NH_2(sym)}$	3272 s, msp	3265 s, msp	3255 vs, msp	3270 s, sp	3280 s, msp	3260 s, msp
δ_{NH_2}	1630 s, sp	1604 vs, msp	1600 vs, msp	1600 vs, msp	1604 s, sp	1600 s, msp
$\nu_{C=N}$	1538 s, msp	1510 s, mbr	1505 ms, mbr	1525 ms, mbr	1532 ms, mbr	1512 s, mbr
ν_{C-S}	608 w, msp	615 ms, sp	615 s, sp	615 w, mbr	614 ms, msp	615 s, mbr
ν_{M-N}	—	310 s, msp	322 w, mbr	312 w, br	318 w, mbr	298 ms, mbr
ν_{M-X}	—	325 s, msp	235 w, mbr	330 w, mbr	250 w, mbr	322 ms, mbr

Band assignment	ZnL_2Br_2	CdL_2Cl_2	CdL_2Br_2	CdL_2I_2	HgL_2Cl_2	HgL_2Br_2	HgL_2I_2
$\nu_{NH_2(asym)}$	3430 3330 s, sp	3375– 3335 s, br	3330 s, br 3375 sh	3330 s, msp 3375 sh	3355 s, msp	3330 s, br	3370 s, msp
$\nu_{NH_2(sym)}$	3280 s, sp	3270 s, msp	3265 s, msp	3260 s, msp	3265 s, msp	3255 s, br	3280 ms, sp
δ_{NH_2}	1613 s, sp	1605 s, msp	1600 s, mbr	1597 s 1618 sh	1628 s, msp	1625 s, br	1631 s, sp
$\nu_{C=N}$	1520 s, sp	1518 s, mbr	1512 s, mbr	1510 s, mbr	1535 s, mbr	1532– 1520 s, br	1538 s, msp
ν_{C-S}	610 ms, mbr	610 s, mbr	608 s, msp	600 s, mbr	612 s, msp	602 s, mbr	618 w, mbr
ν_{M-N}	299 w, mbr	315 w, mbr	310 w, mbr	300 w, mbr	612 s, msp	602 s, mbr	618 w, mbr
ν_{M-X}	240 w, mbr	260 w, msp	—	—	255– 270 w, br	—	—

at 3365 and 3275 cm^{-1} assigned to N–H asymmetric and symmetric stretching modes respectively. The asymmetric stretching band gets split and broadened in some of the complexes and appears in the 3420–3330 cm^{-1} region, whereas the symmetric stretching mode suffers a negative shift of only 7–17 cm^{-1}. Normally the asymmetric stretching frequency suffers a shift to lower frequency on complexation of the $-NH_2$ group. The positive shift may be due to hydrogen bonding in the ligand in the solid state, which is reduced in the complexes. The δ_{NH_2} of the ligand appears at 1630 cm^{-1} as a strong band, which shows a negative shift of 30 cm^{-1} in the complexes. The $\nu_{C=N}$ of the ligand at 1538 cm^{-1} suffers a decrease in frequency (6–33 cm^{-1}) as well as intensity in the complexes, indicating involvement of the ring nitrogen in coordination. The C–S stretching frequency of the ligand is at 608 cm^{-1}, but in the complexes this appears at higher frequencies, by about 10 cm^{-1}. This is also opposite to the generally observed change to lower frequencies on complexation. Therefore we propose that the ligand is linked through the ring nitrogen atom. A medium intensity band corresponding to ν_{M-N} is observed in the 322–294 cm^{-1} region in these complexes.

However, in the case of mercury complexes the IR spectra show a different pattern. The asymmetric N–H stretching frequency shows a large negative shift of about 10–30 cm^{-1}, whereas the symmetric one shows only a small, 7–17 cm^{-1} negative shift. The bending N–H mode remains unshifted but gets slightly broadened. In contrast to the situation in the other complexes, the C=N stretching mode is negligibly affected in the Hg complexes; therefore bonding through ring nitrogen is less likely. Similarly ν_{C-S} also shows a negligible shift, indicating non-participation of ring sulphur in coordination. We therefore tentatively propose bonding of the metal through the exocyclic amino nitrogen in the Hg complexes. A weak intensity band in the 270–255 cm^{-1} region is attributed[2] to ν_{Hg-Cl}.

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ANNOUNCEMENTS

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The congress will be held under the aegis of the Indian Society of Developmental Biologists and is co-sponsored by the Indira Gandhi National Open University.
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INFLUENCE OF ANIONS ON THE ACTIVITY OF PIG PANCREATIC ALPHA-AMYLASE

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ABSTRACT

Pancreatic α-amylase activity has been found to be inhibited by sodium fluoride, ammonium chloride, sodium sulphate, sodium nitrate and sodium bromide. The inhibition was reversible and noncompetitive in nature; each anion produced a substantial decrease in the maximum attainable velocity without having any detectable effect on the Michaelis–Menten constant. The values for 50% inhibition (I_{50}) were: sodium fluoride, 0.25 mM; ammonium chloride, 2.3 mM; sodium sulphate, 3.0 mM; sodium nitrate, 4.7 mM; and sodium bromide, 7.2 mM. The apparent inhibition constant (K_i) for ammonium chloride was found to be 0.5 mM. These observations provide additional evidence that salts alter enzyme activity by altering the organized structure of the protein.

INTRODUCTION

MOST studies of α-amylase have been focused on the amylase of mammalian digestion. Microbial and animal enzyme extracts have been used over many years as supplement in enzyme deficiencies of the pancreas and small intestine. Mammalian α-amylases are therefore of great importance. Anions play a vital role in the growth and development of tissues. The Hofmeister lyotropic series of ions describes the order of effectiveness of ions in influencing a very large number of chemical and physical properties of proteins. The series provides an ordering of both anions and cations for effectiveness in causing denaturation, in influencing activity coefficient, and in leading to structural transition in macromolecules[1]. It has been recently demonstrated that neutral salts at high concentration inhibit the activity of widely different enzymes[2]. Several heavy metal ions have been shown to inhibit mammalian amylases[3]. The crystalline α-amylase of fungal and pancreatic origin is inactivated by many high molecular ammonium quaternary salts, cationic dyes and sodium lauryl sulphate[4]. Several anions, like NO_3^-, Cl^-, Br^-, I^- and thiocyanate, increase the rate of salivary amylase inhibition[5]. Ammonium ion has been reported as an inhibitor of α- and β-amylase from rice. The extent of inhibition has been reported[6] to be 7 to 42%. In our studies on pancreatic α-amylase, the enzyme showed specific anion inhibition, which was strikingly noncompetitive. In this communication we report the effect of five salts, viz. sodium fluoride, sodium bromide, ammonium chloride, sodium nitrate and sodium sulphate on the activity of pancreatic α-amylase. Such studies have not been carried out earlier on pancreatic amylase.

MATERIALS AND METHODS

Pancreatic α-amylase was isolated and purified from pig pancreas according to the method of Fischer *et al.*[7] The enzyme was homogeneous with respect to size and charge as indicated by gel electrophoresis. All chemicals used were of analytical grade. Amylase activity was measured by using dialysed starch as substrate and measuring the amount of reducing sugar liberated by the method of Bernfeld[8]. Protein concentration was determined by the method of Lowry *et al.*[9] The inhibitory activity of anions was determined by incubating different concentrations of the respective salts (0–10 mM) with pancreatic amylase for 10 min, and measuring residual enzyme activity at 540 nm in a Hitachi spectrophotometer, model 200-20. The stoichiometry of anion binding was determined by plotting the data according to Johnson *et al.*[10], in the form of a $\log(V_0 - V_i)/(V_i)$ vs $\log(I)$ plot. The nature of inhibition was determined by the method of Lineweaver and Burk[11]. The apparent inhibition constant (K_i) was determined from a Dixon plot[12], with two different substrate levels (1 to 2%) and various inhibitor concentrations.

RESULTS AND DISCUSSION

Only sodium fluoride (NaF) had a pronounced inhibitory effect at the lowest concentrations (70%

Anions LB [illegible]

at 1 mM). For each anion, the value of 50% inhibition (I_{50}) was read from the curves shown in figure 1. The following I_{50} values for the different anions were calculated: sodium sulphate, 3.0 mM; sodium nitrate, 4.7 mM; sodium bromide, 7.2 mM; sodium fluoride, 0.25 mM; and ammonium chloride, 2.5 mM. In each case, the anion inhibition was found to be reversible and could not be released by increasing the concentration of the substrate. The stoichiometry of anion binding was determined by plotting the data in the form of a $\log(V_0 - V_i)/(V_i)$ vs log (I) plot. Such a plot for sodium fluoride is shown in the inset of figure 1. The plots for the other anions were similar. In each case the relationship was linear. The slope was close to 1.0 for all the five anions studied, suggesting that cooperativity was not present[13]. Ammonium chloride had no effect on the Michaelis–Menten constant, but caused significant decrease in the maximum attainable velocity, V_{max} of the enzyme. These observations suggest that ammonium chloride inhibition is noncompetitive (figure 2). The apparent inhibition constant (K_i) for pancreatic amylase was found to be 0.5 mM.

Data on the effect of different salts on pig pancreatic α-amylase are given in table 1. Ammonium salts were more effective in inhibiting the amylase.

Meur and De[14] studied the activity of human salivary amylase in the presence of anions, and the effect of anions on the pH optimum of the enzyme. Alpha-amylase activity was determined in Tris–anion buffer at pH 6.0, 6.9 and 7.0, and in 0.02 M phosphate buffer at pH 6.9 in the presence of various salts. At pH 6.0, different Tris–anion buffers had little effect on amylase activity. However, at pH 7.0, the activity was markedly affected by anions (aspartate < acetate < phosphate < oxalate < chloride)[14].

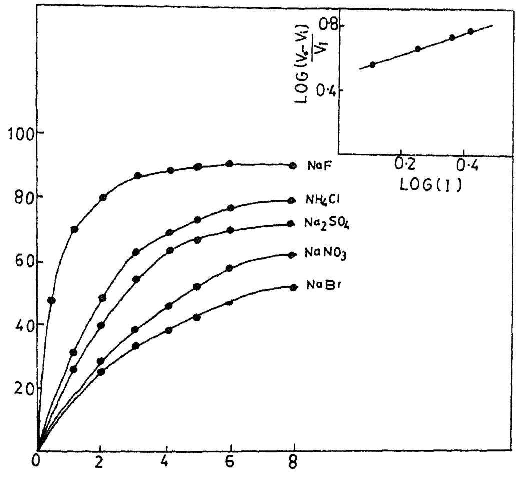

Figure 1. Inhibition of pig pancreatic α-amylase by anions at pH 6.9. Inset shows the plot of $\log(V_0 - V_i)/(V_i)$ vs log (NaF).

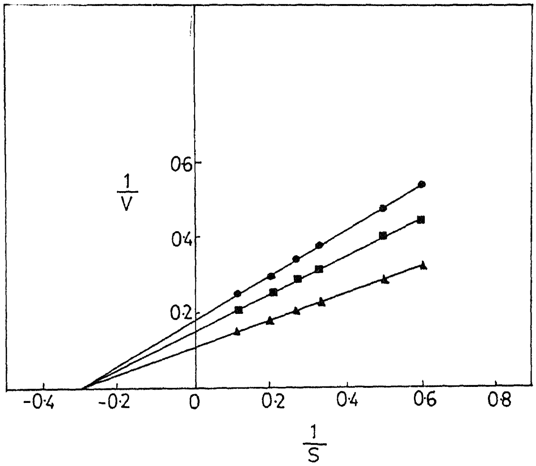

Figure 2. Lineweaver–Burk plot showing noncompetitive type of inhibition of pig pancreatic amylase by ammonium chloride. [●——● control, ■——■ 1 mM NH_4Cl, ▲——▲ 2 mM NH_4Cl.]

Table 1 *Inhibition by different salts on activity of pig pancreatic α-amylase*

Salt	Inhibition (%) 0.2*	0.4	0.6	0.8	1.0
Ammonium persulphate	20	40	50	100	100
Ammonium acetate	10	30	40	56	70
Ammonium ceric sulphate	65	100	100	100	100
Ammonium oxalate	20	75	50	75	100
Molybdic acid	25	35	61	70	71
Phosphomolybdic acid	75	51	91	95	100
Ammonium hydrogen sulphate	25	81	55	72	100
Ammonium sulphate	21	54	60	100	100

*Concentration, in mM.

It has long been recognized that mammalian α-amylase requires chloride for maximum activity. Muss observed, with salivary amylase, that 1–10 mM NaCl gave maximum activity[15], and that the chloride ion depressed the solubility of the enzyme but enhanced its stability against inactivation by heat and by heavy metals. The optimum NaCl concentration for porcine pancreatic α-amylase is 10 mM, with higher concentration producing an inhibitory effect. It has been demonstrated[2] that neutral salts at high concentration inhibit the activity of widely different enzymes in the order, of increasing effectiveness, Ac^-, Cl^-, NO_3^-, Br^-, I^-, SCN^-, ClO_4^-. The inhibition caused by neutral salts at high concentration is due to structural changes in the enzyme molecule. Such changes could be mediated by changes in solvent structure or could result from direct effects on the protein molecule. The inhibition of enzyme activity by neutral salts is probably associated with disruption of enzyme structure demonstrable at salt concentrations where partial activity remains.

Mediation of salt-induced changes in macromolecular structure by change in the organized structure of water has been suggested by Klotz[16]. Jencks[17] has interpreted on the basis of insensitivity of protein disruption to the nature of alkali cations. Robinson and Jencks[18] have presented evidence that salt effects are due to a direct action on peptide and amide groups, or possibly relocation of excluded ions at the polar–nonpolar surface, which would account for cation insensitivity[18].

It appears that the responsible mechanism is structure disruption of enzyme, resulting in accessibility of groups, which, in the absence of salts, are buried and hence nonreactive.

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ANNOUNCEMENT

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Current Science, August 20, 1989, Vol. 58, No. 16 907

AUTOSOMAL DOSAGE COMPENSATION IN INTERSPECIFIC HYBRIDS OF *DROSOPHILA*: TRANSCRIPTIONAL ACTIVITY IN DIPLOID AND TRIPLOID HYBRIDS

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ABSTRACT

Transcriptional activity of a triploid-2L segment has been compared in an interspecific hybrid (I) (compound-2L of *Drosophila melanogaster* ♀ × *D. simulans* ♂) and an intraspecific hybrid (II) (compound-2L of *D. melanogaster* ♀ × Oregon R^+ ♂). The results reveal that although one of the three homologues in hybrid I has been contributed by *D. simulans*, the segment 21A-22F of 2L shows a level of transcriptional activity equal to that in hybrid II, where all the three homologues are from *D. melanogaster*. In the asynapsed region of 2L(21ABC) of hybrid I, the transcriptional activity of the two homologues derived from *D. melanogaster* is double that of the single homologue from *D. simulans*, and two-thirds of that of the two homologues of Oregon R^+. The total transcriptional activity of this asynapsed region (21ABC) in hybrid I is equal to that in hybrid II and the diploid Oregon R^+. These results corroborate the existence of autosomal dosage compensation as proposed earlier and strongly suggest that, in contrast to dosage compensation of X-coded products, autosomal dosage compensation operates through suppression of activity per gene at the level of transcription.

INTRODUCTION

The concept of autosomal dosage compensation was introduced by Devlin *et al.*[1] In studies on transcriptional activity of hyperploid autosomal arms (2L and 3L) of *Drosophila melanogaster*[2-5], it was revealed that several hyperploid autosomal segments have the ability to equalize their transcriptional product to that of the corresponding autosomal segments of diploid Oregon R^+ strain flies. In contrast, in haplo-condition, the smallest chromosome, 4, of *D. melanogaster* does not show hyperactivity in comparison with those of diploid sibs, implying that the mechanism of autosomal dosage compensation might be different from that of the haplo-X in male *Drosophila*[5].

The present study was undertaken to examine the transcriptional behaviour of a segment of triploid autosomal arm (2L) in an interspecific hybrid in which two of the three homologues were contributed by *D. melanogaster* female parent and one homologue came from *D. simulans* male parent. When *D. melanogaster* females are crossed with *D. simulans* males, the hybrid progeny are normally all female but they are sterile[6]. However, using the rescue gene (*lhr*)[7], otherwise nonviable male hybrids appear among progeny of the cross.

The results of analysis of transcriptional activity in trisomic-2L hybrids of *D. melanogaster* and *D. simulans* are presented here. This study has some special significance. It has already been proposed that autosomal dosage compensation in *D. melanogaster* is achieved by suppression of activity per gene. In the hybrid of compound-2L strain of *D. melanogaster* and *D. simulans*, some of the regions of the hyperploid arm remain asynapsed, and provide an opportunity to study the transcriptional activity of the chromosomal elements separately and conjointly. This is an ideal situation for examining the transcriptional activity of an autosome in one, two or three doses.

MATERIALS AND METHODS

The following strains were used:

i) Oregon R^+ (wild-type strain of *D. melanogaster*).
ii) C(2L) SHI; F(2R) *bw* (compound-2L strain of *D. melanogaster*).
iii) *D. simulans* (wild type).

Generation of hyperploid strains

To generate the interspecific trisomic-2L strain, *D. melanogaster* C(2L) SHI; F(2R) *bw* females were

Interspecific hybrid; Dosage compensation; Diploid crossed with *D. simulans* males; the progeny arising in F_1 were called hybrid I. The intraspecific trisomic-2L strain (hybrid II) was obtained by crossing C(2L) SH1; F(2R) *bw* females to Oregon R^+ males or vice versa. Both hybrids (I and II) were lethal at the late pupal stage.

The stocks were maintained at 20±1°C in a bacteriological incubator and cultured on sterile *Drosophila* culture medium containing maize powder, agar sugar, propionic acid and Nipagin.

Autoradiography

Salivary glands from the larvae of both hybrids (I and II) and Oregon R^+ were dissected out and immediately transferred into *Drosophila* Ringer containing [^{3}H]uridine (500 μCi/ml, sp. activity 12,000 μCi/mM, obtained from BARC, Bombay) and incubated for 10 min. The procedure for chromosome preparation and processing for autoradiography was as described previously[8]. Photomicrographs and grain counts were obtained on a Zeiss Photomicroscope III.

Grain count analysis

[^{3}H]Uridine-induced silver grains over the 21A-22F region of 2L in both hybrids (I and II) and Oregon R^+ were counted under a 100×(oil-immersion) objective. In all cases, the number of grains on the tip of 2R (59A-60F) were counted for use as internal standard. Besides, grains were counted on a specific region of 2L, viz. 21ABC in hybrid I, as this region remains in an asynapsed

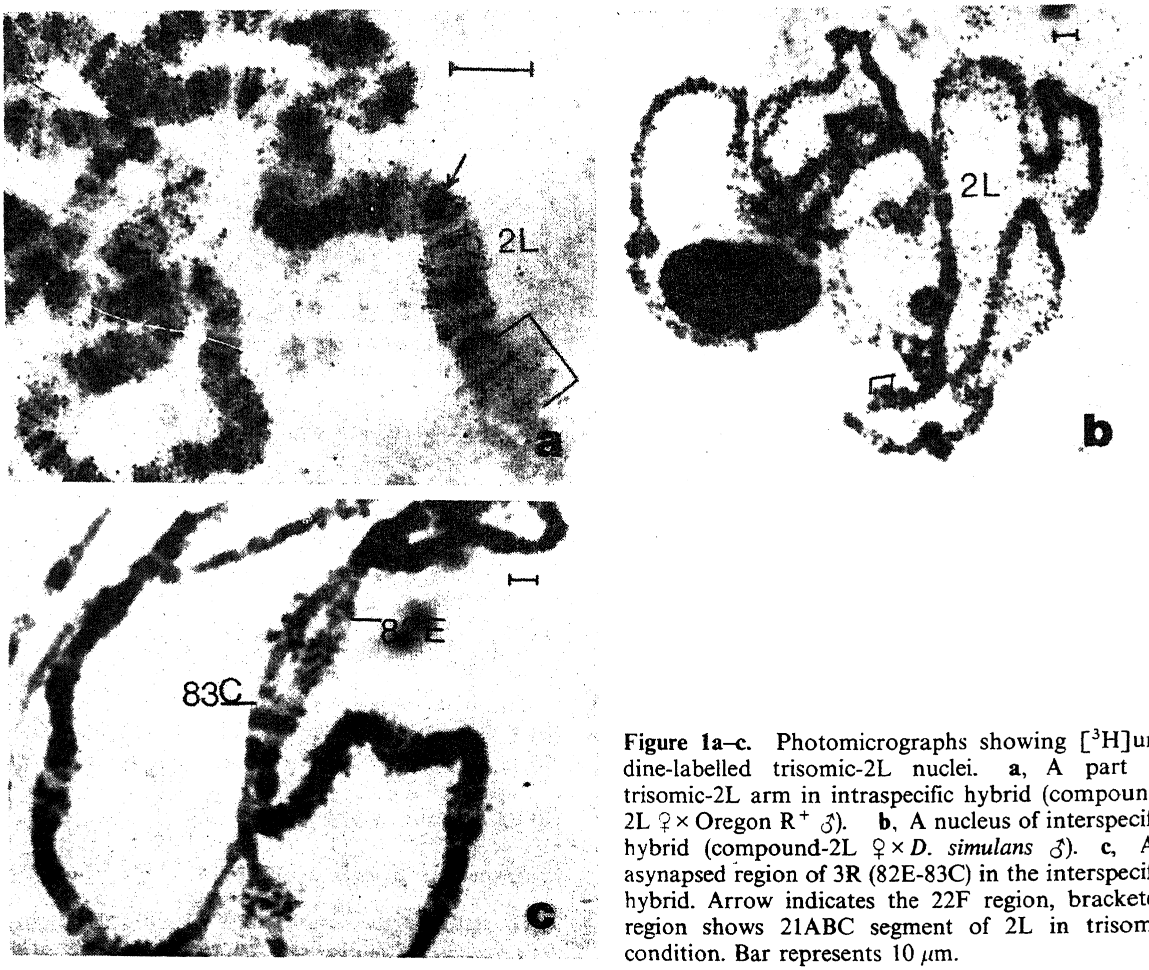

Figure 1a–c. Photomicrographs showing [^{3}H]uridine-labelled trisomic-2L nuclei. **a**, A part of trisomic-2L arm in intraspecific hybrid (compound-2L ♀ × Oregon R^+ ♂). **b**, A nucleus of interspecific hybrid (compound-2L ♀ × *D. simulans* ♂). **c**, An asynapsed region of 3R (82E-83C) in the interspecific hybrid. Arrow indicates the 22F region, bracketed region shows 21ABC segment of 2L in trisomic condition. Bar represents 10 μm.

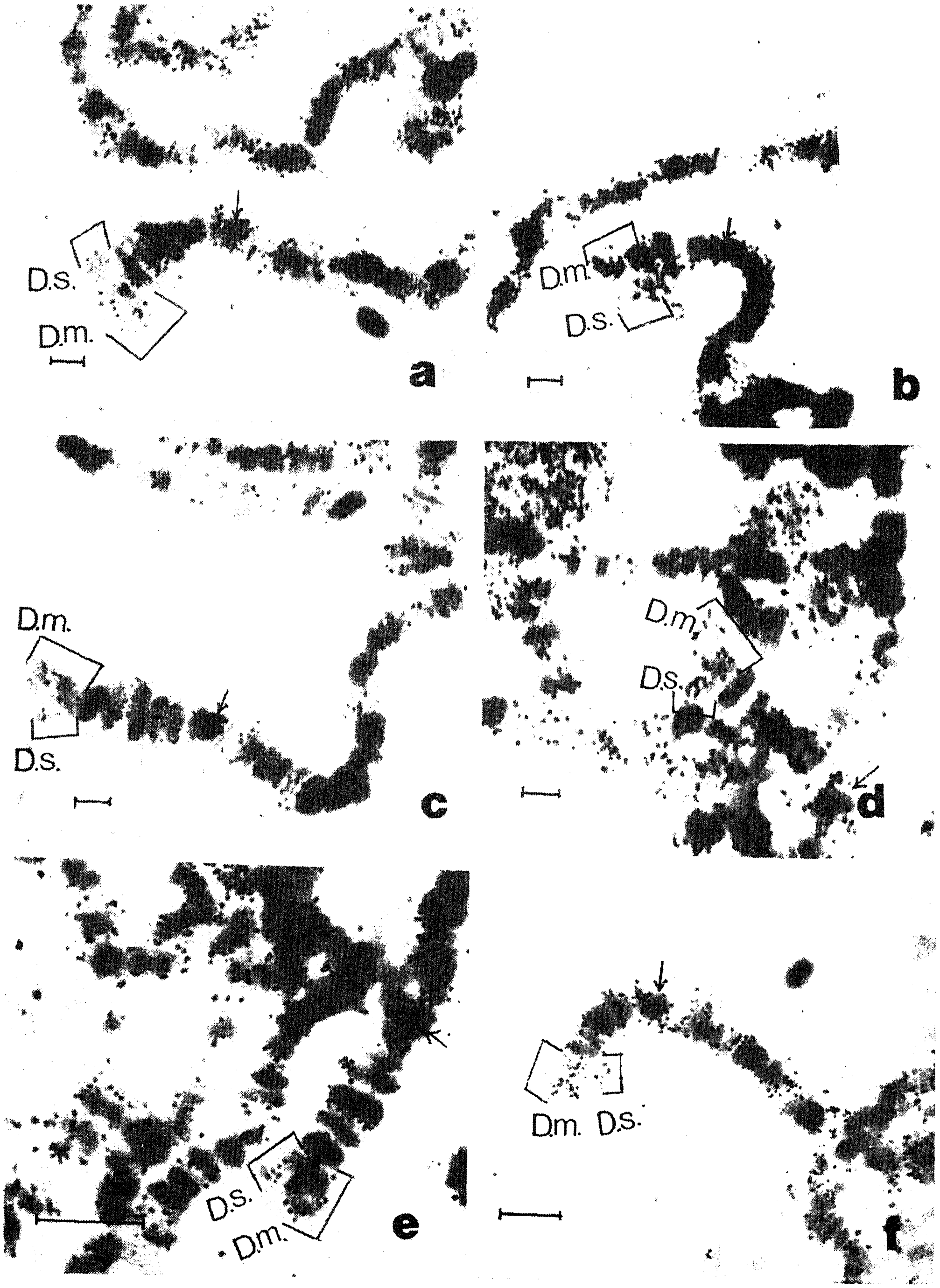

Figure 2a–f. Photomicrographs showing [^{3}H]uridine incorporation in an asynapsed region of 2L (21ABC) in different nuclei of the interspecific hybrid (compound-2L ♀ × *D. simulans* ♂). Bracketed regions show the asynapsed 21ABC region. D.m., *D. melanogaster* homologue; D.s., *D. simulans* homologue.

condition in the majority of nuclei. We also counted the number of [^{3}H]uridine-induced silver grains on the homologues of *D. melanogaster* and *D. simulans* in an asynapsed region (82E-83C of 3R) of a diploid autosomal arm in hybrid I.

RESULTS

In both hybrids (I and II) the hyperploid-2L arm can be distinguished from diploid counterparts by its wider diameter (figure 1a, b). In hybrid I, the tip of 2L (21ABC), i.e. the distalmost segment, remains in asynapsed condition (figure 1b and figure 2a–f).

The mean number of grains on 21A-22F of 2L and 59A-60F of 2R in the two hybrids (I and II) are presented in table 1. The data reveal that although one of the three homologues in trisomic-2L of hybrid I is from *D. simulans*, the 21A-22F region shows a level of transcriptional activity comparable to that of the trisomic-2L of hybrid II, where all three homologues are derived from *D. melanogaster* (21A-22F/59A-60F ratio of grain counts of hybrid II/21A-22F/59A-60F ratio of grain counts of hybrid I = 0.93, table 1). The data in table 2 reveal that in hybrid I the transcriptional activity of the 21ABC region of the two homologues derived from *D. melanogaster* is twice as much as that of the single homologue from *D. simulans* and two-thirds as much as that of the two homologues of Oregon R^{+} (see also figure 2a–f). This finding suggests that transcriptional activity of the two homologues from *D. melanogaster* is at a reduced level in the triploid-2L of hybrid I. On the other hand, the total transcriptional activity of the 21ABC region of the three homologues of hybrid I is equal to that of hybrid II as well as to that of the diploid Oregon R^{+}, suggesting that this region is dosage-compensated in both the hybrids (I and II) at the level of RNA synthesis (table 2).

It is evident from table 2 and figure 1c that in hybrid I the transcriptional activity of the 82E-83C of 3R (asynapsed region) of a single *D. melanogaster* homologue is the same as that of a single *D. simulans* homologue.

DISCUSSION

Studies on whole-arm trisomy of 2L and 3L in *D. melanogaster* suggested that several autosomal segments of a hyperploid autosomal arm synthesize RNA at a level comparable to that synthesized in the diploid condition[3,4]. The present results indicate that the level of activity of the triploid-2L segment of each of the homologues in hybrid I (two derived from *D. melanogaster* and one from *D. simulans*) is similar to that of each homologue of hybrid II, where all three homologues are from *D. melanogaster*. The transcriptional activity of this segment (21A-22F) in the triploid condition (in hybrid I) is shared equally by the two homologues from *D. melanogaster* and the one from *D. simulans*. It is likely that the regulatory sequences involved in transcription of autosomal arms have been conserved in these two species of *Drosophila*. Chatterjee and Ghosh[9] made such a contention while discussing the transcriptional behaviour of the X chromosome in female hybrids of *D. melanogaster* and *D. simulans*. Their results indicated that in the female hybrid, the

Table 1 *[^{3}H]Uridine incorporation in the 2L segment (21A-22F) in hybrids of compound-2L strain and Oregon R^{+} strain of D. melanogaster (hybrid II), hybrids of compound-2L strain of D. melanogaster and D. simulans (hybrid I), and Oregon R^{+} flies*

Strain	Dose (2L)	Mean number of grains ± SE: 2L (21A-22F)	Mean number of grains ± SE: 2R (59A-60F)	Mean T2L/2R ± SE	Ratio of T2L/2R values: HII/HI	Ratio of T2L/2R values: HII/OR	Ratio of T2L/2R values: HI/OR
Compound-2L ♀ × Oregon R^{+} ♂ (HII)	3	122.90 ± 11.76 (11)	100.20 ± 9.94 (11)	1.22 ± 0.017 (11)	0.93	1.01	1.09
Compound-2L ♀ × *D. simulans* ♂ (HI)	3	116.92 ± 12.26 (14)	89.64 ± 10.24 (14)	1.31 ± 0.03 (14)			
Oregon R^{+} (OR)	2	41.58 ± 2.42 (13)	34.76 ± 2.57 (13)	1.20 ± 0.02 (13)			

Figures in parentheses are number of nuclei examined. HII, Hybrid II; HI, Hybrid I.

Table 2 *[^{3}H]Uridine incorporation in the 21ABC region of 2L in hybrid I, hybrid II and Oregon R$^+$*

Strain	Dose (21ABC)	Mean grain ratio of 21ABC and 59A-60F (2R) (21ABC/2R)	Ratio of 21ABC/2R values					Mean grain ratio of 82E-83C and 59A-60F(2R) (82E-83C/2R value ± SE)
			HII/HI	HII/OR	HI/OR	HIa/HIb	HIa/OR	
Compound-2L ♀ × Oregon R$^+$ ♂	3 (all D.m.)	0.48 ± 0.01 (HII) (11)	1.04	0.96	0.92	2.06	0.62	
Compound-2L ♀ × *D. simulans* ♂	3 a.2-D.m. b.1-D.s.	0.31 ± 0.02 (HIa) 0.15 ± 0.01 (HIb) (16)						0.38 ± 0.01* 0.36 ± 0.04* (12)
Oregon R$^+$	2 (all D.m.)	0.50 ± 0.01 (OR) (13)						

D.m., *D. melanogaster* homologue; D.s., *D. simulans* homologue.
Figures in parentheses are number of nuclei examined.
*Transcriptive activity of two homologues of an asynapsed region of 3R (82E-83C) in hybrid I nuclei.

X chromosomes of both *D. melanogaster* and *D. simulans* transcribe equally. In this case, X chromosome transcription is regulated by the combined action of the haploid autosomal complements of each species. Lakhotia *et al.*[10] studied the transcriptional behaviour of the haplo-X chromosome of *D. simulans* in a *D. melanogaster* background. They used the property of unstable transmission of the ring X chromosome of *D. melanogaster* to generate clones of XO cells in the salivary glands of female hybrids. Their results reveal that the single X of *D. simulans* maintains its normal hypertranscriptional behaviour in the *D. melanogaster* background.

It has been contended by earlier workers[2,5,11] that autosomal arms in hyperploid condition show regulation of transcription through a suppression of activity per gene. The present results reveal that transcription from 21ABC in 2L-trisomy is about the same in hybrids I and II and shows compensation for gene dosage when compared with transcription from the same region in diploid Oregon R$^+$ strain flies (see also Ghosh[3]). It is interesting that 21ABC of the two homologues derived from *D. melanogaster* in hybrid I is transcriptionally two-thirds as active as 21ABC of diploid Oregon R$^+$. This finding strongly supports the idea that equalization of gene product in hyperploid condition is achieved by the suppression of activity per gene at the level of transcription[2,5,11].

On the basis of the transcriptional behaviour of the triploid segment in hybrids I and II, it may be suggested that the regulatory sequences involved in autosomal gene regulation are conserved in *D. melanogaster* and *D. simulans* and that autosomal dosage compensation in hyperploid condition is achieved by the suppression of activity per gene rather than by hyperactivation as in the case of haplo-X of male *Drosophila*.

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17 May 1988

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SHORT COMMUNICATIONS

PRECIPITATION OF MgO BY A 'BIO-METHOD'

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ALKALINE earth oxides can be obtained from their organic salts. We have described earlier[1] that MgO can be obtained from magnesium acetate by burning it in excess of air. Carbon, hydrogen and even nitrogen escape as the gases. Sometimes unburnt carbon may make separation of the oxides difficult. This communication reports an easy method of removing the carbon from the organic compounds.

Magnesium acetate was taken as the starting material. It was dissolved in distilled water and was allowed to stand for six weeks. The fungi *Monilinia* and *Fusarium oxysporum* were grown in it. The overall appearance of the fungus in the initial stages was pale yellow. Growth was then rapid and the colour became green with development of fruiting bodies. The final colour was brown-black. The fungus extracted carbon from the acetate. It was observed that the growth rate of the fungus increased (i) when it was exposed to air, and (ii) with dilution, but decreased on addition of MgO powder, Mn salts, etc.

The fungus was filtered out. The clear solution was boiled for a few minutes. On cooling fine particles of MgO settled at the bottom of the container.

The particles of MgO thus obtained had, an average size of $\sim 3\ \mu m$. Chemical analysis indicated that the method produced a very pure material as indicated below:

MgO from magnesium acetate by the burning method: lanthanide impurities, 2.6 ppm; MgO from magnesium acetate by the 'bio-method': lanthanide impurities, 1.2 ppm. Reflectivity of the sample was 11% better than that of MgO (AR) from BDH.

Another advantage of this method is that we can infer completion of the process when the third stage (see above) of the fungus is reached. As no acetate can precipitate out from the solution only those particulates (MgO) precipitate out that have lost their carbon structures.

The MgO obtained had an absorption maximum at 213 nm and had a luminescence response similar to that of fine-grained MgO described elsewhere[2]. MgO obtained by burning the acetate has a different surface state, which is responsible for 2.3 eV emission[1]; the samples obtained by the present method give 2.9–3.1 eV emission.

It may be noted that no reactants are involved in this method and hence the purity of the final product can be as good as the purity of the starting material. As purity of organic compounds can be achieved to a high degree, we may expect that the method is a powerful one for obtaining high-purity MgO and other alkaline earth oxides.

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VERTICAL DISTRIBUTION OF SPECTRA OF HEAT AND MOMENTUM FLUXES OVER EAST AND WEST COASTS OF INDIA DURING MONEX—1979

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SPECTRAL analysis has been widely used to measure dominant periodicities in meteorological parameters and to understand the causes of the oscillations.

A short-range periodicity of nearly 5 days in different meteorological parameters has been revealed by several investigators[1-4] and is related to the frequency of the monsoon 'lows' and depressions travelling from the Bay of Bengal to North India. Mishra[5] observed a westward propagating pressure wave of 4–5-day period in the tropics. Cadet[6] observed a 10–12-day periodicity in precipitable

Fungal growth; carbon
interaction

water and meridional wind fields, and inferred that these fluctuations appeared to be associated with the westward propagating mode north of the equator.

In the present study an attempt has been made to look for possible periodicities in eddy sensible heat flux, eddy latent heat flux and eddy momentum flux at each level in the vertical from 1000 mb/surface to 300 mb surface, with intervals of 50 mb, over the east and west coasts of India during MONEX-1979 (May through August, 00 GMT). The spectral calculations have been made in the eddy sensible heat transport, eddy latent heat transport and eddy momentum transport[7]. The observed periodicities are classified into three groups, viz. short range (2–5 days), medium range (above 5–10 days) and long range (10–30 days).

Figures (1 and 2) show the vertical distribution of periodicities in eddy sensible heat, eddy latent heat

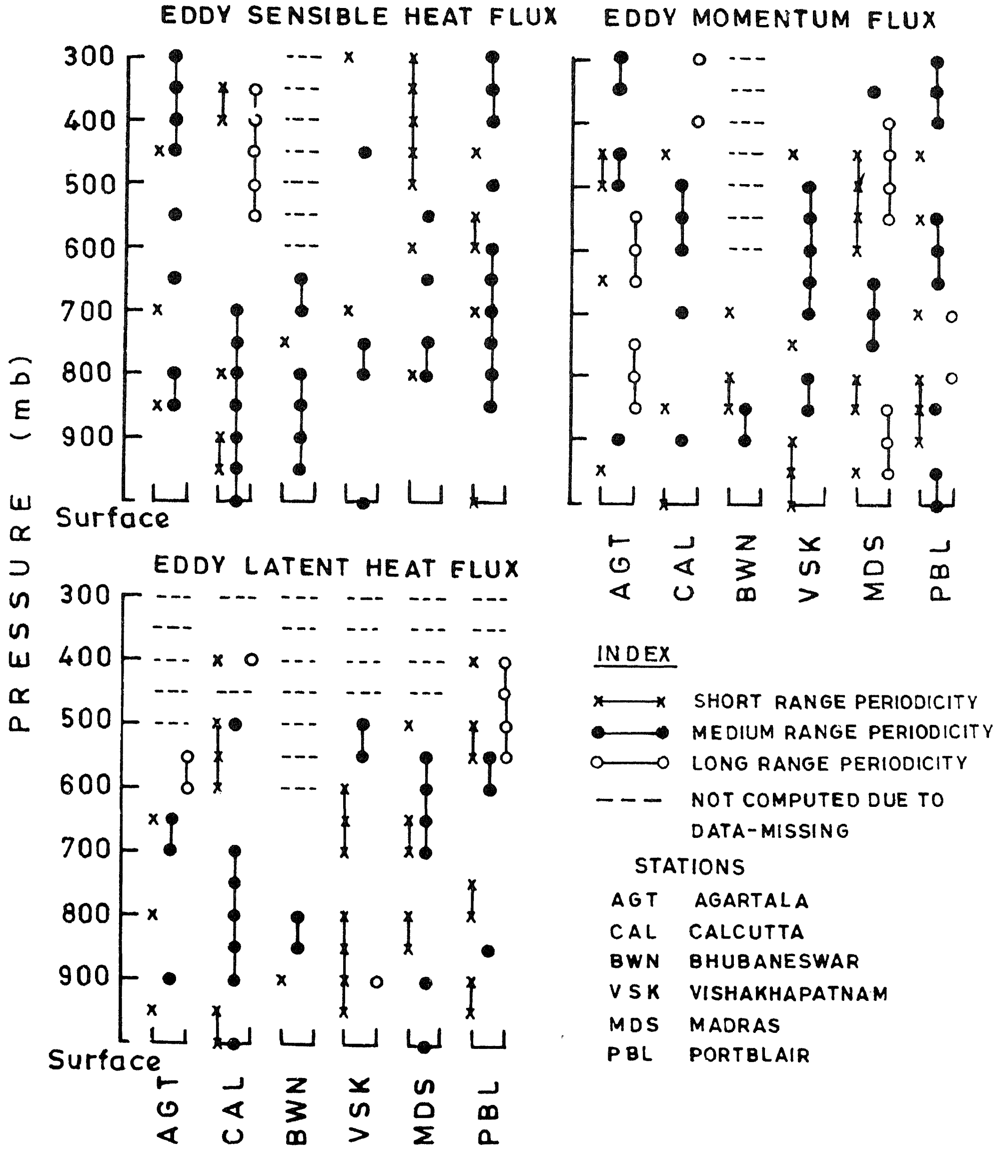

Figure 1. Vertical distribution of periodicity over east coast of India.

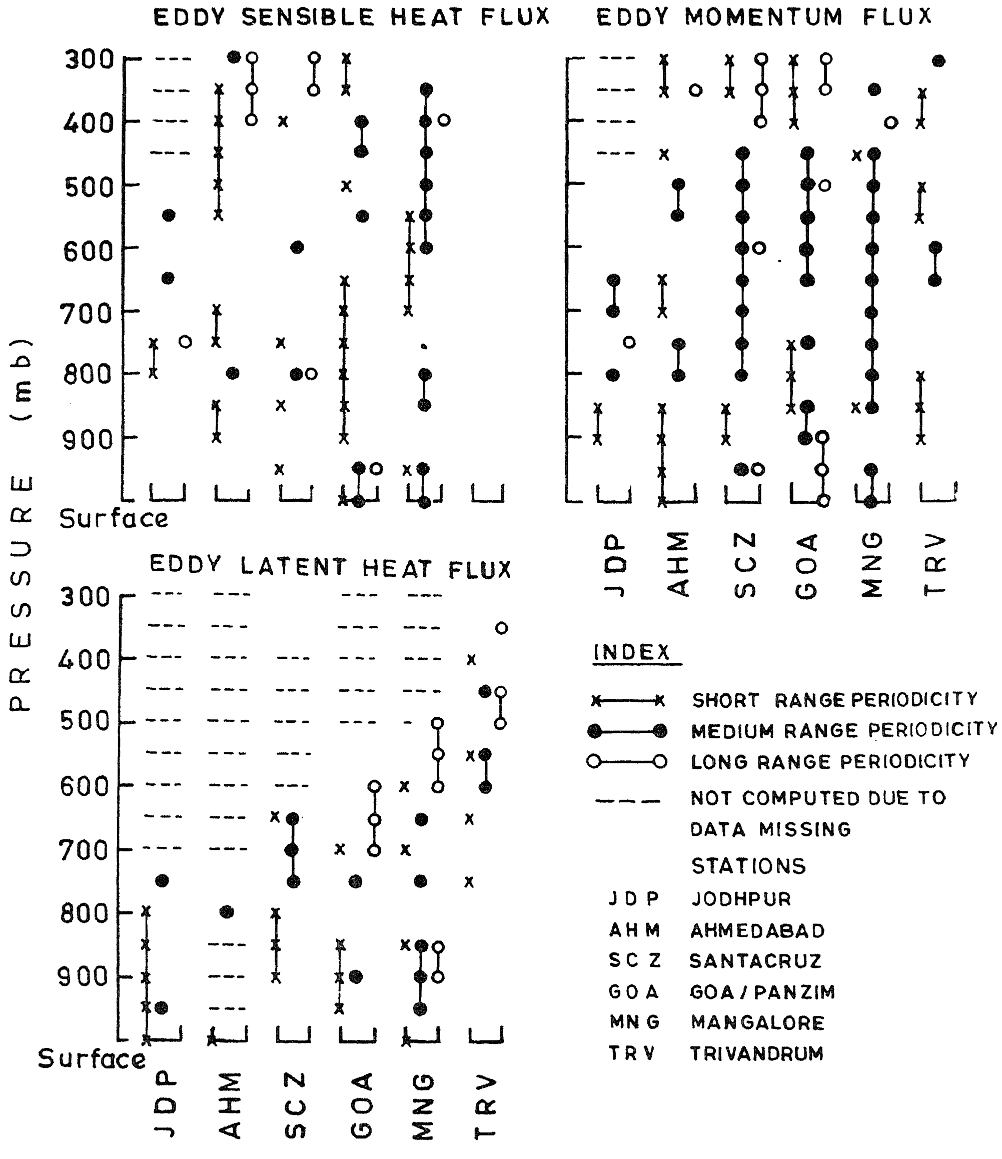

Figure 2. Vertical distribution of periodicity over west coast of India.

and eddy momentum transport, over east and west coasts of India respectively. It is seen that the long range periodicity is usually present in the eddy sensible heat and eddy momentum fluxes above 400 mb surface, between Ahmedabad and Goa. This long range periodicity is also observed below 900 mb surface over Santacruz and Goa in eddy momentum and eddy sensible heat transport. Between 900 and 400 mb surfaces, the existence of long range periodicity in eddy sensible heat and eddy momentum transport is rarely observed over west coast of India. Thus, the long range periodicity in eddy sensible heat and eddy momentum fluxes appears to be more pronounced at upper pressure levels above 400 mb surface between Ahmedabad and Goa and less pronounced below 900 mb surface. The eddy latent heat transport shows the presence of long range periodicity between Goa and Trivandrum in the middle as well as the lower troposphere.

The eddy momentum transport is dominated by medium range periodicity between 900 and 400 mb surfaces over west coast of India. However, the eddy sensible heat transport is dominated by short range periodicity over west coast between 900 and 400 mb surface, whereas at Mangalore the medium range periodicity in eddy sensible heat transport is equally effective.

The long range periodicity has been observed in eddy sensible heat transport at Calcutta between 550 and 350 mb surface, in eddy latent heat transport at Agartala between 550 and 600 mb and at Port Blair between 400 and 550 mb surface and in eddy momentum transport between 500 and 650 mb as well as between 750 and 850 mb surface at Agartala and at 300 mb and 400 mb surfaces at Calcutta and at Madras between 400 and 550 mb surface as well as below 700 mb at Madras and Port Blair. Thus, long range periodicity is usually observed over the extreme north of the east coast of India in the middle and the upper troposphere in eddy sensible and latent heat transport and in the lower and middle troposphere in eddy momentum transport. However it is observed at Madras and Port Blair in the middle troposphere in eddy latent heat transport and in the lower and middle troposphere in the eddy momentum transport. The short and medium range periodicities are well distributed throughout the east coast in eddy transport of sensible and latent heat as well as momentum.

Finally, we conclude that short and medium range periodicities are present throughout the east and west coasts of India between 1000 and 300 mb surface with different fluxes. The long range periodicity is relatively infrequently observed in lower and upper troposphere.

One of the authors (RKS) thanks UGC, New Delhi, for a fellowship.

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EPR SPECTROSCOPIC STUDIES OF CALCIC-PLAGIOCLASES FROM THE ARCHAEAN ANORTHOSITES OF HOLENARASIPUR, KARNATAKA CRATON, SOUTH INDIA

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THIS communication presents a study of the structural state of calcic-plagioclases from the terrestrial Archaean anorthosites of the Holenarasipur belt in Karnataka Craton, South India, by an electron paramagnetic resonance (EPR) spectroscopic study of Fe^{3+} ions substituting for Al^{3+} tetrahedral sites in the structure. The six-point Rb/Sr whole-rock isochron data provided by Kutty *et al.*[1] for the Holenarasipur anorthosites indicate that they are 3095 Ma old and are of Archaean age.

In the three representative EPR spectra of the 23 calcic-plagioclases studied from the 3095-Ma-old Archaean anorthosites, we observed the Fe^{3+} spectral component occupying the distorted tetrahedral sites, indicating the disordered structural state of plagioclases attained during the crystallization event, similar to that of lunar plagioclases. In the study of lunar plagioclase Weeks[2] not only observed the Fe^{3+} signal at $g = 4.27$ in the EPR spectrum but also recognized Ti^{3+} signal at $g = 1.98$. We have been able to ascribe the absence of Ti^{3+} spectral component in the plagioclase of Holenarasipur anorthosite to the presence of titanium in the tetravalent state based on the recognition of Ti^{3+} signal at $g = 1.998$ in the EPR spectrum of plagioclase heated to 650°C under reducing condition.

EPR spectra of Fe^{3+} ions in Ca-plagioclases

EPR first-derivative spectra were taken at 78 K on a Varian E-109 E-line X-band spectrometer at about 9.2 GHz. The phase-pure powder samples examined weighed about 30 mg. Three representative EPR spectra of the 23 plagioclase samples studied from the Archaean anorthosites of Holenarasipur are presented in figure 1 and are interpreted.

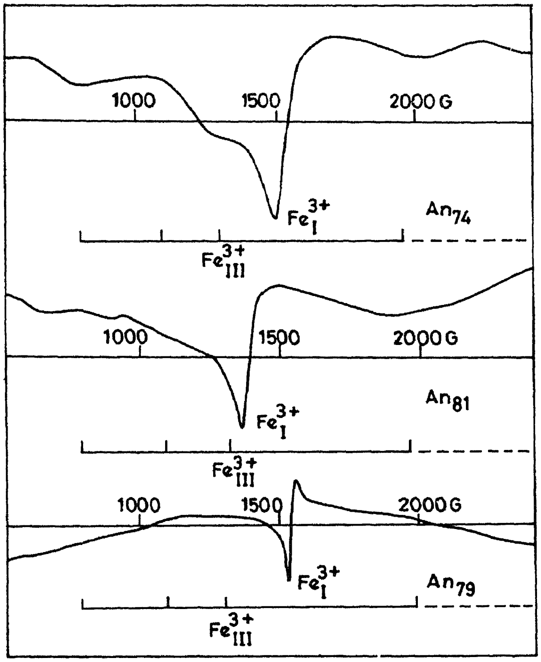

Figure 1. Typical EPR spectra for Fe^{3+} in plagioclases from Holenarasipur anorthosites (February, 9.2 GHz; temperature, 78 K).

EPR studies of Lesnov *et al.*[3] on calcic-plagioclases and of Hofmeister and Rossman[4] show that the number of sites for Fe^{3+} ions depends on structural state rather than on plagioclase chemistry. The linear correspondence of EPR double-integrated intensities with optical peak areas shows that all Fe^{3+} ions are tetrahedrally co-ordinated in both calcic-plagioclases and disordered K-feldspars. As the Fe^{3+} ions enter the plagioclase structure substituting for the Al^{3+} ions, Fe^{3+} follows Al^{3+} as regards the Al–Si distribution among the tetrahedral sites. Based on this it has been possible to use EPR of Fe^{3+} for investigating order–disorder relations in calcic-plagioclases. The different types of Fe^{3+} sites existing in plagioclases are found to be related to the structural state. This was established[3] by a study of 150 calcic-plagioclases from the gabbro-anorthosite suite of rocks. The three different types are: Fe^{3+}(I) with effective g-factor near 4.3; Fe^{3+}(II) with $g_1 = 4.5$, $g_2 = 3.84$ and $g_3 = 3.65$; Fe^{3+}(III) with $g_1 = 6.88$, $g_2 = 5.04$, $g_3 = 3.69$, $g_4 = 3.48$ and $g_5 = 2.2$.

Figure 1, which shows three typical EPR spectra of powdered calcic-plagioclases from Holenarasipur, also shows the relative intensities, linewidths and g-values of the three different Fe^{3+} sites. The characteristic features of these EPR spectra are described.

The EPR spectrum of specimen An_{74}, exhibits signals representative of site Fe^{3+}(III), displaying broad linewidth and lower signal intensity compared to signals of sites Fe^{3+}(I) and Fe^{3+}(II). This type of spectrum is observed only in one specimen.

The EPR spectrum of specimen An_{81} shows broad linewidth and very low intensity for Fe^{3+}(III) in contrast to Fe^{3+}(I). This can be attributed to Fe^{3+} occupying distorted tetrahedral sites converging into a complex site in calcic-plagioclase structure.

The EPR spectrum of specimen An_{79} actually comprises a sum of a complex Fe^{3+}(I). This complex set Fe^{3+}(I) is indicative of increasing disorder of the Fe^{3+} surroundings, as interpreted by Lesnov *et al.*[3] Their detailed investigations show how Fe^{3+} ions substituting for Al^{3+} in various sites in calcic-plagioclases cause variations in their structural state.

It can be inferred that EPR spectroscopic studies of plagioclases from the Archaean anorthosites of Holenarasipur reflect their disordered structural states due to Fe^{3+} ions occupying the distorted tetrahedral sites in the structure.

EPR spectrum of Ti^{3+} in plagioclase

In the EPR spectrum of lunar plagioclases, unlike those from the plagioclases of terrestrial Archaean Holenarasipur anorthosites, Weeks[2] observed not only the Fe^{3+} signal at $g = 4.27$ but also recognized the spectral component due to Ti^{3+} at $g = 1.98$.

In order to account for the absence of Ti^{3+} spectral component in the plagioclases of Holenarasipur anorthosites, EPR spectra of terrestrial plagioclase samples (i) unheated, (ii) heated under oxidizing condition, and (iii) heated under reducing condition were examined (figure 2). The Ti^{3+} spectral component shows up only in the plagioclase heated under reducing condition. The absence of Ti^{3+} spectral component in the unheated sample and in the sample heated under oxidizing condition can be ascribed to the presence of titanium in the tetravalent state in plagioclase.

The absence of Ti^{3+} spectral component in the terrestrial Archaean anorthosites of Holenarasipur indicates that the plagioclase crystals were formed under oxidizing conditions, unlike those of lunar plagioclases, which must have crystallized under reducing conditions.

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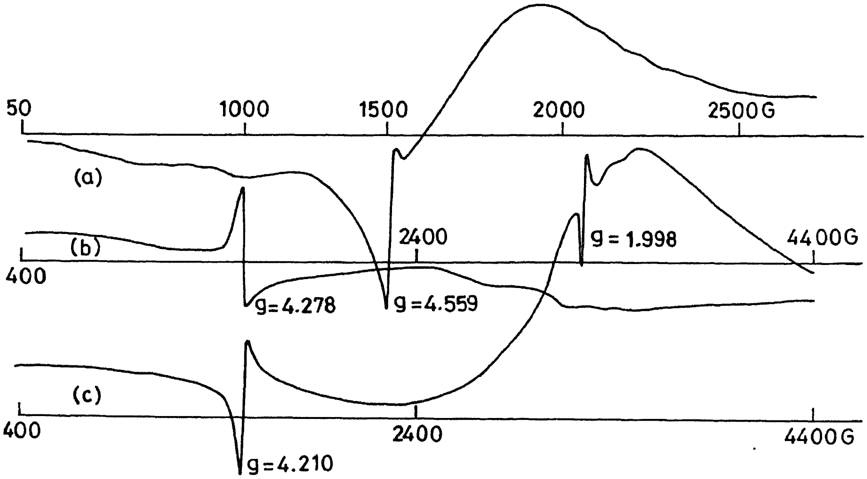

Figure 2. EPR spectra of a plagioclase from Holenarasipur (frequency, 9.2 GHz; temperature 78 K. (a) Untreated sample, (b) Oxidized at 650°C, (c) reduced at 650°C.

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A NEW SPECIES OF *SCYTALIDIUM* FROM INDIA

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DURING our studies on litter decomposition of *Tectona grandis* in the forests of Madhya Pradesh a new species of *Scytalidium* was isolated.

On microscopic examination and comparison, the present fungus was found to be distinct from the three known species of *Scytalidium* in having bigger conidia[1,2]. Therefore the fungus is described here as a new species of *Scytalidium* from India. The specific epithet of this collection has been given after the name of host plant.

Scytalidium tectonae sp. nov. Soni, Jamaluddin and Rajak

Colonies effuse, dark, blackish brown to black; hyphae mid- to dark-brown, smooth, 5.0–7.5-μm thick; conidiogenous cells fragmented and forming arthroconidia; integrated, intercalary conidia of two types—hyaline, thin-walled, cylindrical or oblong, unicellular to bicelled, in chains, 5.0–17.5 × 3.75–5.0 μm in size; thick-walled, oblong, broader, doliform or broadly ellipsoidal, 0- to 3-septate, 7.5–20.0 × 3.75–7.5 μm in size (figure 1).

Coloniae effusae, fuscae ad aterae; cellulae conidigenae fragmentae et formantes arthroconidia; conidia integra, intercelaria, bityporum; conidia determinata, cylindrica, doliiformia, oblonga vel ellipsoida, hyalina, tenuitunicata, unicellularis ad bicellularis, in catenis 5.0–17.5 × 3.75–5.0 μm, conidia crassitunicata, oblongata, doliformia vel late ellipsoida 0–3-septata, 7.5–20.0 × 3.75–7.5 μm.

Culture has been deposited at CMI, Kew, under accession no. IMI 283013 and also in the Regional Forest Research Centre, Jabalpur, India.

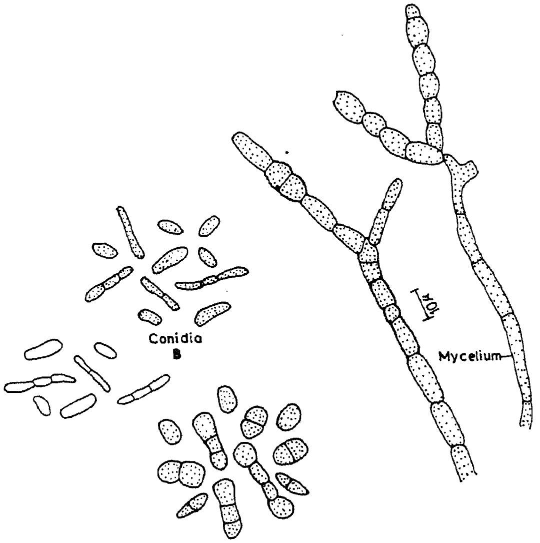

Figure 1. *Scytalidium tectonae.*

Grateful thanks are due to the Director, CMI, Kew, England, for confirmation of the identity of this fungus.

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OCCURRENCE OF WATER-BORNE CONIDIAL FUNGI ON *PINUS ROXBURGHII* NEEDLES

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AQUATIC hyphomycetous fungi, now known as water-borne conidial fungi, predominantly occur on submerged dead leaves of a variety of deciduous dicotyledonous trees[1,2], and are absent or very rarely present on conifer needles[3]. Taxo-ecological work in India indicates that these fungi are unable to colonize submerged conifer needles[4,5].

During a study on aquatic hyphomycetous fungi of the Kumaun Himalaya, samples of grey-black submerged needles of *Pinus roxburghii* from a freshwater stream, the Niglat, were collected from different spots in separate polythene bags. These needles were washed thoroughly under running tap-water and finally rinsed in sterile distilled water to remove extraneous sediments and invertebrates. The leaves were cut into small pieces and incubated in petri dishes containing sterilized stream water at room temperature (15–20°C). On alternate days the needles were examined for the presence of water-borne conidial fungi.

A total of nine species belonging to eight genera of water-borne conidial fungi were observed. Among these, *Lunulospora cymbiformis* Miura and *Triscelophorus monosporus* Ingold were found to be dominant over other species. The conidia of *Clavariopsis aquatica* de Wild., *Lunulospora curvula* Ingold, *Flabellospora verticillata* Alasoadura, *Tetrachaetum elegans* Ingold, *Tricladium splendens* Ingold, *Campylospora chaetocladia* Ranzoni and *Alatospora acuminata* Ingold were found to be less common in occurrence.

The occurrence of such a large number of species of these fungi at a time on *P. roxburghii* needles suggests their involvement, hitherto unreported, in the decomposition of *P. roxburghii* needle litter in freshwater habitats.

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A NEW HOST RECORD FOR *ALTERNARIA ALTERNATA* (FR.) KEISSLER AND ITS TOXIGENIC POTENTIAL

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Ocimum basilicum L. a medicinal plant growing wild in the university campus, was found to develop a leaf spot disease during the rainy season of 1986. The symptoms were circular to irregular necrotic lesions of 5–8 mm diameter, sometimes with concentric rings. The lesions coalesced to form large, irregular necrotic patches in severe cases. The pathogen was identified as *Alternaria alternata* following the characteristics given by Ellis[1]. This is the first report of its occurrence on *O. basilicum*. Since several metabolites produced by *Alternaria* species have been associated with phytotoxicity[2], an attempt was made to test the toxigenic potential of *A. alternata* and to identify the compounds responsible for phytotoxicity.

Culture filtrates of the fungus cultured in Czapek–Dox broth for 30 days were employed for testing phytotoxicity as reduction in germination of seeds and elongation of roots of tomato and also on detached leaves of tomato and *O. basilicum*. The toxic metabolites produced by the fungus were extracted with ethyl acetate and tested for phytotoxicity after separation by TLC as described earlier[3].

The reduction in germination of seeds and elongation of roots in treated samples was as high as 85% and 94% respectively compared with the control. Detached leaves of tomato and *O. basilicum* when treated with the culture filtrates exhibited epinasty with inward rolling of the leaf lamina. TLC analysis of the culture filtrates revealed the presence of two phytotoxic compounds: a brown-coloured compound, formed an elongated spot on the TLC plate with an R_f value extending from 0.25 to 0.36, and another compound with an R_f of 0.49, emitted blue fluorescence when exposed to UV light. UV spectral characteristics[4], colour reactions with ethanolic ferric chloride[5] and *p*-anisaldehyde[6], besides cochromatography with authentic sample, confirmed the identity of the brown-coloured compound as tenuazonic acid.

Although species of *Alternaria* produce many toxic metabolites, only tenuazonic acid is listed in the registry of toxic effects of chemical substances[7] and its mycotoxic nature has also been well established in recent years[8]. Natural occurrence of tenuazonic acid in blast-affected rice plants[9] and tomato paste[6] was also reported. As the leaves of *O. basilicum* are widely used to cure bowel complaints in children, headache and dullness of hearing[10], it is important to test the occurrence of toxic metabolites in infected plants.

The authors thank Dr P. M. Scott, Health Protection Branch, Canada, for providing a sample of tenuazonic acid. Financial assistance from CSIR, New Delhi, to MV is gratefully acknowledged.

5 November 1988; Revised 26 December 1988

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AN UNRECORDED POST-HARVEST DISEASE OF APPLE IN KUMAUN HILLS

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The Kumaun hills are known for fruit and vegetable production. Apple is one of the main fruit crops of

Toxins;

this region. The economy of this region mostly depends on apple cultivation. A number of pathogens are responsible for spoilage of fruits in transit, storage, market and plantation[1–5]. During a survey on pre- and post-harvest diseases of fruit crops, a fruit rot of apple variety Golden Delicious, incited by *Cordana musae* (Zimm.) Höhnel during storage was detected. This disease of apple has not hitherto been reported from India.

A survey was conducted during 1986–87 in the four main fruit-growing areas of Kumaun region, viz. Bhowali, Chaubattia, Champawat and Mukteshwar. The diseased fruits collected in polythene bags from orchards, markets and storage were either used immediately or stored at 10°C. The causal organism was isolated and purified according to the method of Tandon and Bilgrami[6]. Morphological study and identification of the fungus were done[7,8]. Cultures of the pathogen were maintained on potato dextrose agar. Grangner and Horne's[9] method was used for pathogenicity test. Twenty healthy fruits of apple variety Golden Delicious were inoculated with the test pathogen and incubated at 25 ± 2°C for 10 days. Disease symptoms and cultures were studied to establish Koch's postulates.

Disease symptoms appear as water-soaking necrotic lesions on the fruit surface and the fruit turns soft as the disease advances. The pathogen damages the mesocarp tissue, which turns into a brown to black mass of fungal hyphae and spores. In advanced stages of the disease, the whole mesocarp region undergoes decay and the pathogen sporulates enormously. The seeds of the mature fruit are also infected and show a wrinkled surface covered with spore mass (figure 1).

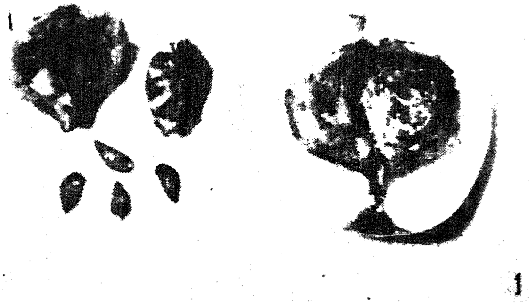

Figure 1. Fruit showing infection in mesocarp and on seeds.

Colonies on agar are greyish-brown, with erect conidiophores, reaching up to 4 to 5 cm in diameter within a week. Conidiophore straight, up to 210 μm long and 4–7 μm thick, usually wider at the base (7–10 μm). Conidia solitary, produced apically or subapically, obovate, 1-septate, rounded at the tip and pointed at the base.

In India, this pathogen has been reported on banana leaves from Coimbatore, causing large, oval, pale-brown spots[10]. The pathogen was isolated for the first time from diseased apple fruits in the Kumaun hills. In storage, a single infected fruit may be the source of spoilage of fruits in considerable amounts. Various kinds of injuries facilitate the entry of the pathogen during harvesting, packing and transportation.

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CONTROL OF CITRUS GREENING DISEASE IN INDIA

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GREENING disease is one of the major causes of decline in citrus orchards in India. The disease is caused by a bacterium-like organism[1] and transmitted by a psyllid vector, *Diaphorina citri*[2]. Attempts have been made to control this malady by the use of certain tetracycline antibiotics[3,4] and Penicillin G[5]. However, these antibiotics have not been found effective in management of the disease in the field. Here we report work in which the effectiveness of a chemical, CPAN No. MJN 1891, under patent in the USA, has been determined in the field and glasshouse.

In the first experiment 12 trees of Malta sweet orange (*Citrus sinensis*) of 10 to 12 years age growing in the orchard of the Indian Agricultural Research Institute, New Delhi, were selected on the basis of symptoms and categorized visually as showing mild, intermediate and/or severe greening symptoms. The experimental trees were indexed for greening before and after eight months of treatment. The trees were also observed for general appearance and health. Suitable control trees were identified and were also indexed against greening on the selected indicator host (Darjeeling orange, *C. reticulata*)[6].

A hole of 25 mm deep and 15 mm in diameter was made in the trunk with a brace and bit. One gram of CPAN No. MJN 1891 (formulation in petroleum jelly) was inserted in the hole with the aid of a clean glass rod. The hole was then sealed with aluminium foil and cellophane tape to make it air-tight and to prevent drying of the compound. All the 12 trees were treated in the way described above. Control trees received no treatment but had identical holes in their trunks filled with petroleum jelly only.

In the second experiment, two-year-old glasshouse-infected seedlings of mosambi (*C. sinensis*) were selected for chemical treatment. Six plants each were sprayed for about two min until runoff with CPAN No. 1891 in concentrations of 500 and 1000 ppm. Three sprays, two weeks apart, were given. Control plants were sprayed with water only.

In the third experiment, eight mosambi plants infected with greening disease were treated by inserting 100 mg of the chemical beneath the bark by giving a clean cut of about 3 cm length in the bark and resealing it. Two plants were used as controls.

The chemical was found to be effective against infection in trees showing mild and intermediate greening symptoms (table 1). The treated trees showed improvement in general appearance and more fruits matured on them. However, severely affected trees did not show significant recovery in respect of either symptoms or fruit bearing. Control, untreated trees showed signs of greater deterioration.

In the second experiment, two of the six plants treated with 1000 ppm CPAN No. MJN 1891 recovered after six months but the chemical caused leaf-tip scorching and unusual curling. Three of the six plants treated with 500 ppm of the chemical showed complete recovery (absence of any disease symptoms) and no phytotoxic effect. Indexing of recovered plants six months after remission of symptoms failed to indicate the presence of pathogen. One plant treated with 500 ppm concentration showed symptoms again after one year.

Table 1 *Results of CPAN No. MJN 1891 treatment of citrus trees for control of greening disease*

	No. of fruits matured		
Tree no.	Before treatment	After treatment	Back indexing
1*	06	22	−
2**	28	36	+
3*	05	16	−
4**	14	08	+
5**	21	08	+
6*	09	18	−
7**	02	00	+
8*	36	67	−
9*	20	58	−
10*	12	26	−
11 & 12 Died			
Controls			
1**	00	00	+
2**	02	00	+
3**	32	21	+
4**	16	07	+

+, Greening reaction on Darjeeling orange; −, no reaction on Darjeeling orange.
*Flowered normally in following years without visible disease symptoms: fruit setting near normal.
**Trees further deteriorated; flowered heavily but without fruit setting.

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*For correspondence.

In the third experiment, all the eight plants treated showed complete recovery while the untreated ones showed decline in growth, and absence of development of any new flush of foliage in spring, as was the case in the treated plants. The two untreated plants appear to be on the verge of collapse and death.

Full recovery of six out of eight treated trees originally having mild to moderately severe greening infection indicates high effectiveness of the chemical. This is also reflected in the overall healthy appearance of the treated trees and increase in fruit yield. However, severely affected trees did not show marked recovery or improvement in vigour. During the same period, deterioration of the control trees was more pronounced. The glasshouse experiment shows that spraying of the chemical is less effective and phytotoxicity at higher doses was an additional disadvantage in this mode of application. Reappearance of symptoms in one of the recovered plants indicates that the plant was not completely cured by spray application.

The method of direct insertion of the chemical was found to be the most suitable one as the treated plants recovered fully and reappearance of greening symptoms did not occur. This was further confirmed by indexing.

The effectiveness of the chemical CPAN No. MJN 1891 in controlling greening disease of citrus has been demonstrated. Since the pathogen is restricted to the phloem region, it is essential that chemical application be so designed that the chemical reaches the phloem region or the conducting tissues directly and in the shortest possible time. These studies will be helpful in developing greening-free nucleus planting material and if this material is grown in the vector-free areas already identified[7], it will form a crucial component of the certification programme.

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NATURAL AUTOFLUORESCENCE IN OOSPORES OF *PERONOSCLEROSPORA SORGHI* AND THEIR VIABILITY

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PERONOSCLEROSPORA SORGHI (Weston & Uppal) C. G. Shaw is a major problem for the production of sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) in many parts of the world[1,2]. The pathogen produces two types of spores, viz. conidia and oospores. The oospores are mainly responsible for perpetuation of the pathogen through unfavourable seasons[3]. There are several reports on oospore germination in *P. sorghi*[2,4].

According to Williams[2] the subjects of the longevity, viability and germination of oospores of the graminaceous downy mildews are characterized by confusion and contradiction. Time and again the need was emphasized for an improved method for determining oospore viability in graminaceous downy mildews to meet the inadequacies in the previous methods[2,5]. An inverse relationship between natural autofluorescence in fungal spores and their viability was reported by Wu and Warren[6]. The present study was undertaken to determine viability of the oospores of *P. sorghi* using fluorescence microscopy.

Oospores of *P. sorghi* were collected from infected and dried leaves of sorghum by the method of French and Schmitt[7] and stored at room temperature (25 ± 5°C). One-year-old oospores were plated for germination on water agar in petri dishes[4,7]. After 3–4 days, a portion of water agar along with oospores was removed using a cork borer, mounted on a microscope slide and covered with a coverglass, and observed for the presence/absence of autofluorescence. The experiment was repeated thrice and 400 oospores were observed each time.

A Leitz Orthoplan epifluorescence system with a

Germination

Pleomopak fluorescence vertical illuminator was used to observe the oospores for autofluorescence. The light source was a 200 W high-pressure mercury lamp (HBO 200). A Leitz Wetzlar Fluota lens was used to enhance the brightness of the fluorescence at wavelengths of 450–490 μm.

Photomicrographs were taken with a normal 35 mm Leitz Vario-Orthomat camera system using ASA 400 fast film (ORWO NP 27) and 1–2 min exposure time. To show the relationship between fluorescence and viability, comparable pictures of bright field and fluorescence were taken of the same field.

Examination of the material plated for germination revealed that 56% of the oospores emitted fluorescence (figure 1). The percentage of oospore germination was 8. The germinated oospores did not show fluorescence in the protoplasm (figure 2).

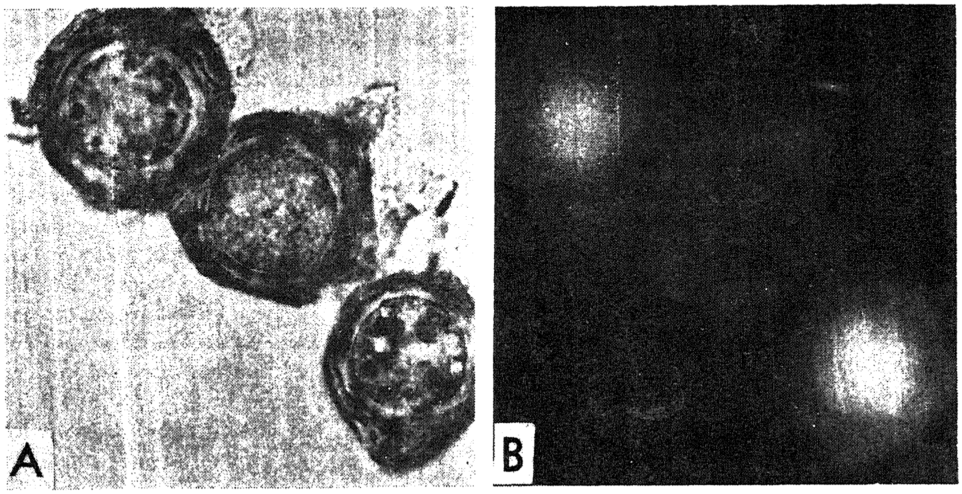

Figure 1. Oospores of *Peronosclerospora sorghi* mounted in water (× 750) under (**A**) bright-field and (**B**) fluorescence microscopy.

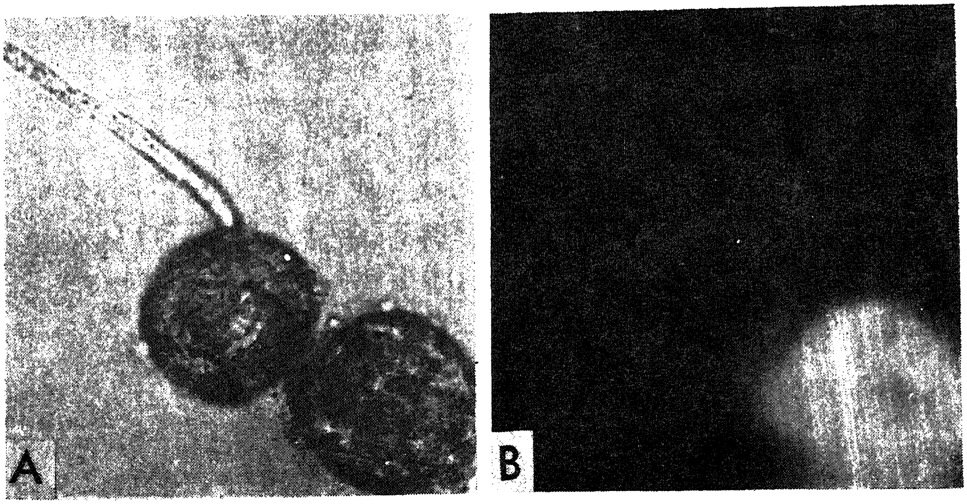

Figure 2. Germinated and ungerminated oospores of *Peronosclerospora sorghi* on water agar (× 750) under (**A**) bright-field and (**B**) fluorescence microscopy.

To examine the possibility of staggered germination, oospores sown on water agar with furfural/seedlings were removed after 3–4 days from the agar surface, rinsed 8–10 times in sterile distilled water, and then transferred to petri dishes containing water agar with furfural/seedlings.

In the test for staggered germination, up to 2% of the oospores germinated when they were plated for the second time on water agar with furfural/seedlings.

Non-fluorescent but non-germinated oospores of *P. sorghi* are in a state of dormancy. The occurrence of staggered germination of oospores confirms this view. Kaveriappa[8] also suggested that all the viable oospores do not germinate simultaneously.

Wu and Warren[6] observed natural autofluorescence in the cytoplasm of conidia, pycnidiospores, ascospores and mycelia of 12 species of fungi incubated on agar plates, soil or in suspension. The authors concluded that natural autofluorescence in fungal materials indicated their death, and found a relationship between autofluorescence and viability. The same procedure is useful for determining viability of the oospores of *P. sorghi*. The technique may be suitable for determining viability of other downy mildew fungi.

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IN VITRO GROWTH OF SOME FUNGI ISOLATED FROM WHEAT PHYLLOPLANE IN RELATION TO SO_2 TREATMENT

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SULPHUR dioxide adversely affects microbes on aerial plant surfaces and suppresses the pathogenicity of many plant pathogens[1]. A number of field observations have been made on the effects of SO_2 on saprophytic and parasitic micro-organisms with special reference to diseases of plants grown in SO_2-polluted localities. Singh[2] observed that the population of some test fungi inoculated on the phylloplane of wheat decreased on prolonged exposure to SO_2 at $2669 \pm 105\ \mu g/m^3$ concentration. However, little is known about effects of SO_2 on growth of phylloplane fungi. The present communication deals with effects of SO_2 on growth of some phylloplane fungi *in vitro*.

The experimental system was designed according to the method described by Magan and Lacey[3]. The following fungi, isolated from the phylloplane of wheat, were selected for the study: *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Drechslera australiensis*, *Epicoccum purpurascens*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *P. citrinum*. Mycelial blocks (5 mm each) cut from actively growing margins of colonies of the fungi were transferred separately onto petri plates, in triplicate, containing 20 ml of potato dextrose agar (PDA) medium. The lids of the plates were raised slightly using sterile paper clips, and the plates containing the mycelial blocks were placed in a fumigation chamber. Dishes of glycerol/water were placed in the bottom of the chamber to provide required humidity. The petri plates were exposed to sterilized air–SO_2 mixture for 10, 30 and 60 min, with the concentration of SO_2 adjusted to $2669 \pm 105\ \mu g/m^3$ air. Plates exposed to gas-free air served as control. The plates were incubated at 24 ± 2°C after the treatment and growth was measured after 24 h of incubation. Percentage growth stimulation/inhibition was cal-

Table 1 *Effect of sulphur dioxide (2669 ± 105 μg/m^3), expressed as per cent inhibition/stimulation of growth, on some phylloplane fungi*

	Exposure time (min)		
Species	10	30	60
A. alternata (Fr.) Keissler	−9.25 ± 0.58*	−15.13 ± 0.33**	−22.68 ± 0.67**
A. flavus Link ex Fries	+2.09 ± 0.67	−4.20 ± 0.33*	−8.39 ± 0.33**
A. niger van Tiegham	+4.41 ± 0.58*	−1.96 ± 0.33	−10.78 ± 0.67**
C. cladosporioides (Fresen.) de Vries	−6.05 ± 0.33	−13.64 ± 0.58**	−19.69 ± 0.88*
C. lunata (Wakker) Boedijan	−1.76 ± 0.33	−5.29 ± 0.67*	−10.60 ± 0.67**
D. australiensis (Bugnicourt) Subram. and Jain	−3.00 ± 0.67	−10.45 ± 1.15*	−17.90 ± 0.67**
E. purpurascens Ehrenb. ex Schlecht.	+7.79 ± 0.33*	−5.22 ± 0.33	−7.79 ± 0.33*
F. oxysporum Schlechtendahl	+1.74 ± 0.33	−3.05 ± 0.58*	−7.86 ± 0.38*
P. chrysogenum Thom	−13.70 ± 0.33*	−23.52 ± 0.58*	−29.41 ± 0.33**
P. citrinum Thom	−7.71 ± 0.58	−15.41 ± 0.33**	−19.94 ± 0.33**

All values are mean ± SE.
+, Stimulation; −, inhibition.
**t* significant at $P = 0.05$; ***t* significant at $P = 0.01$.

culated as [Per cent stimulation/inhibition = (Difference in colony diameter (mm) of test species between control and treated/colony diameter of control) × 100].

Colony growth of all the test fungi was significantly ($P = 0.05$) inhibited on prolonged SO_2 exposure (table 1). Growth of *A. alternata, C. cladosporioides, C. lunata, D. australiensis, P. chrysogenum* and *P. citrinum* was inhibited by all three durations of exposure, and the inhibition was more significant ($P = 0.01$) for the 60 min exposure (table 1). The maximum inhibition was of *P. chrysogenum* and the minimum, *E. purpurascens*, for the longest exposure time. *A. flavus, A. niger, E. purpurascens* and *F. oxysporum* showed growth stimulation at the shortest exposure time (10 min). Per cent growth inhibition of all the test fungi increased with increasing exposure time. In general, the order of inhibitory effect of SO_2 was *P. chrysogenum* > *A. alternata* > *P. citrinum* > *C. cladosporioides* > *D. australiensis* > *A. niger* > *C. lunata* > *A. flavus* > *F. oxysporum* > *E. purpurascens* (table 1).

It is reported that SO_2 inactivates the sulphhydryl groups of enzymes[4]. Inhibition of acid phosphatase activity by SO_2 has been reported[5]. The increased colony growth of some of the test fungi after short exposure may be due to their resistance and ability to neutralize the toxic effects of SO_2 or because of their ability to use SO_2 as nutrient source to a certain extent.

Financial help provided by CSIR, New Delhi, is thankfully acknowledged.

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A NEW RECORD OF DIPLOID CYTOTYPE OF *SCHIZANDRA GRANDIFLORA* HOOK. F. & THOMS. FROM INDIA

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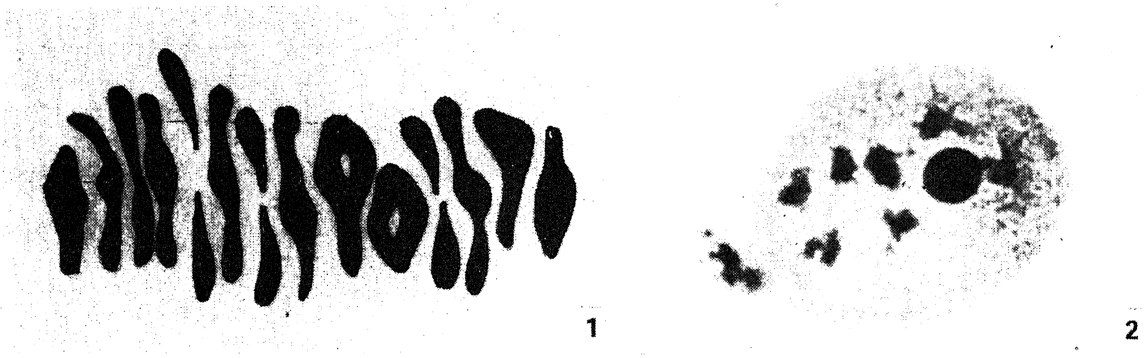
SCHIZANDRA GRANDIFLORA (family Schizandraceae), common in dense forests, is a large, glabrous, woody climber with unisexual, white, fragrant axillary flowers. Of the six species of *Schizandra* reported from India[1], *S. grandiflora* is the only species found in the Western Himalaya between 1900 and 3100 m

Cytology



Figures 1 and 2. Chromosomes of *S. grandiflora*. **1**, PMC showing 14 bivalents at metaphase I (× 2640). **2**, PMC showing 7 bivalents at diakinesis (× 1260).

altitude. For the present studies, material was collected from different locations. Young floral buds were fixed in Carnoy's fluid for 24 h and then transferred to 90% ethyl alcohol. Meiotic preparations were made usually by squashing the developing anthers in 1% acetocarmine. Vouchers are preserved in the Herbarium, Department of Bio-Sciences, HPU, Shimla.

Cytological investigations revealed that meiosis was perfectly normal in all the individuals studied. A chromosome number of 14 bivalents at diakinesis and metaphase-I (figure 1) was obtained for the material collected from Shimla (2350 m), Theog (2350 m) and Shilaru (2500 m), while 7 bivalents were recorded at diakinesis (figure 2) and metaphase-I for the material collected from Baghi (2800 m). Normal bivalent formation with equal segregation of chromosomes has been observed in both the cytotypes (diploid and tetraploid). The average pollen fertility for the diploid is 94% and for the tetraploid 97%. There is no significant difference in the morphology and size of the pollen grains between the cytotypes.

The present count of $n=14$ agrees with the previous report[2,3] of $2n=28$. The diploid cytotype with $n=7$ has been reported for the first time from India. However, its existence in Nepal has already been reported[4].

The diploid plant is short compared to the tetraploid plants. By and large, the diploid cytotype can be distinguished in the filed by its large but thin and light-coloured leaves compared to the tetraploid cytotype.

Of the three species studied so far, all are tetraploid with $n=14$. The intraspecific diploid with $n=7$ has been recorded for *S. grandiflora* only. So the genus seems to be monobasic with $x=7$ as the base number.

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11 October 1988; Revised 17 February 1989

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PLANT REGENERATION FROM POLYETHYLENEGLYCOL ADAPTED CALLUS OF RICE

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THE use of *in vitro* techniques akin to the selection of variants in bacterial and fungal cultures has been proposed and employed in very few higher plants[1-3]. Plant cell culture methods allow selection of variants, such as those resistant to water stress[4,5], which would have practical application in agriculture.

The present communication deals with the successful isolation of rice calli adapted to 10, 20, 30 and 50 g of polyethyleneglycol (PEG, mol. wt 6000) and subsequent regeneration of plants. PEG induces water stress in the medium by acting as a non-penetrating osmotic agent that lowers the water potential of the medium. Callus cultures of *Oryza sativa* L. cvs. Tellahamsa and Sureka (susceptible to water stress) were initiated from mature embryos on Linsmaier and Skoog's[6] (LS) medium containing 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2% sucrose. All the cultures were incubated under continuous, cool white fluorescent light (2000 lux) at 26±2°C. Callus cultures were maintained on the same medium for six subcultures of 25-day duration (control). Callus tissues of both cultivars were then transferred to LS medium supplemented with 2 mg/l 2,4-D, 2% sucrose and different concentrations of PEG (10, 20, 30 and 50 g/l).

Healthy callus masses were isolated and subcultured on the respective media for six passages of 25 days each. Callus cultures of cv. Sureka growing on LS medium without PEG (control) and on 20 g/l of PEG are shown in figures 1A and B. Calli growing on different concentrations of PEG appeared healthy and yellowish compared to control (without PEG) calli, which were slightly brown. Regenerating ability of all the callus cultures was tested (figure 2) on LS basal medium containing 1 mg/l indole-3-acetic acid, 4 mg/l kinetin, 10 g/l PEG and 2% sucrose. Callus cultures of both cultivars developed green spots during the first 20 days of culture on the LS basal medium. A second passage on the same medium but without PEG was necessary for successful whole-plant regeneration from these tissues.

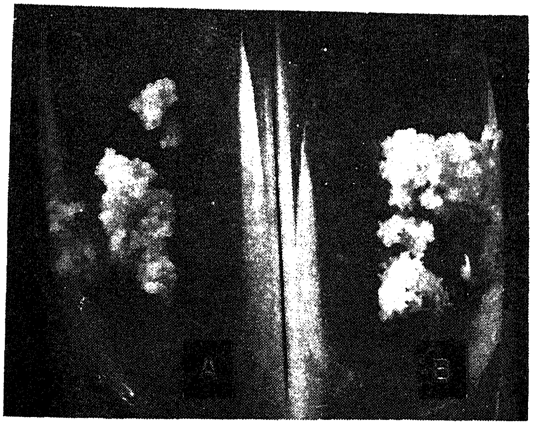

Figure 1A, B. Callus of rice cultivar Sureka on LS medium (**A**) without PEG (control), and (**B**) containing 20 g/l PEG.

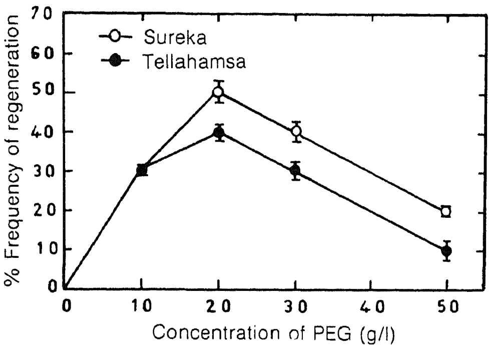

Figure 2. Regenerating ability of rice cultivar Sureka (○) and Tellahamsa (●) callus cultures adapted to different concentrations of PEG. Data are average of 10 replicates ±SE.

Callus cultures of both cultivars maintained on medium without PEG (control) however failed to give any organogenetic response after 12 passages (300 days). Plantlet regeneration from a 20 g/l PEG-adapted callus of cv. Sureka is shown in figure 3.

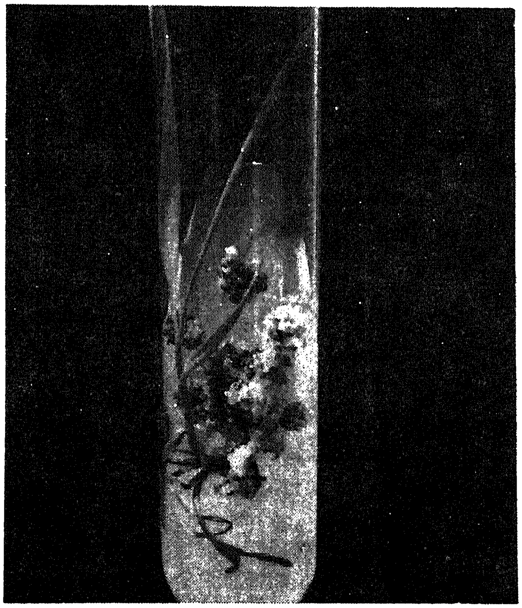

Figure 3. Regeneration of plantlets from 20 g/l PEG-adapted callus of rice cultivar Sureka.

Regenerating ability of the calli was not very significant (figure 2) in the two cultivars used. The frequency of plant regeneration was lowest in 50 g/l PEG-adapted calli of both cultivars. The plants were transferred successfully to pots for further evaluation. Sievert and Hildebrandt[7] observed variations among tobacco cells for their ability to grow on different carbon sources. Clones that vary in their ability to produce anthocyanin[8] and tropane alkaloids[9], and to grow in the absence of plant growth regulators[10] have been isolated earlier. This shows that within a population of cells, individual cells with different phenotytic characteristics or with altered metabolism also exist. The change in colour of the PEG-grown and calli of rice cultivars may suggest the occurrence of an adaptation process or an epigenetic phenomenon in the cells.

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PROTECTION OF MUNG BEAN SEEDLINGS AGAINST HEAT SHOCK BY A SUBSTITUTED PHTHALIMIDE

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ESSENTIALLY all crops are adversely affected by high temperatures, especially during critical stages of plant development. Where this occurs, it is important that crops develop a certain degree of thermotolerance to cope with the temperature assault. Our knowledge about acquisition of thermotolerance in plants by chemical growth regulators is extremely limited. Exogenously applied gibberellic acid (GA_3) has been shown to enhance thermotolerance of mung bean seedlings[1]. A recently synthesized group of substituted phthalimides has been shown to mimick GA effects, but it is not known whether these compounds could also show a protective response against heat shock. The present study has investigated the effect of a substituted phthalimide AC 94,377 on the heat shock response of etiolated mung bean seedlings.

Etiolated 48-hour-old mung bean seedlings (*Vigna radiata* L. Wilczek. var. ML-131) with an axis length of 2 cm, grown at 28°C, were incubated in 1 mM phosphate buffer, pH 6.0, containing 1% sucrose at appropriate temperatures. In order to study the effect of the substituted phthalimide AC 94,377 (1-(3-chlorophthalimido)-cyclohexanecarboxamide), it was added to phosphate buffer and either applied to seedlings as a pretreatment at 28°C (2 h) or 40°C (2 h), or its application was continued during exposure to the heat-shock temperature of 45°C (3 h). A concentration of 100 μm was found to be the best. After the treatment the seedlings were grown on water in germination paper rolls at 28°C in the dark for 72 h. Length of whole seedlings (root plus hypocotyl), hypocotyls and primary root were then measured.

Compared with the growth of normal seedlings grown entirely at 28°C, the growth of seedlings given a 3-h 45°C treatment was severely inhibited (table 1). Hypocotyl growth was inhibited by 58% and primary root growth by 52%. After a pretreatment at 40°C for 2 h, the seedlings became more thermotolerant. Compared with the heat-shocked seedlings, in pretreated seedlings hypocotyl growth was enhanced by 57% and root growth by 83%. Whe-

Table 1 *Effects of temperature and a substituted phthalimide AC 94,377 on acquisition of thermotolerance in mung bean seedlings*

Treatment	Hypocotyl length (mm)	Primary root length (mm)	Whole seedling length (mm)
28°C (5 h)	95^a	75^a	170^a
28°C (2 h), 45°C (3 h)	40^d	36^e	76^f
40°C (2 h), 45°C (3 h)	63^c	66^b	129^d
40°C + Phthalimide (2 h), 45°C (3 h)	66^c	64^b	130^d
40°C + Phthalimide (2 h), 45°C + phthalimide (3 h)	83^b	68^b	151^b
28°C + Phthalimide (2 h), 45°C (3 h)	65^c	50^d	115^e
28°C + Phthalimide (2 h), 45°C + phthalimide (3 h)	80^b	60^c	140^c

Values are means of 20 readings. Means within the same column with the same letter in the superscript are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

applied during the 40°C pretreatment, phthalimide did not significantly enhance the thermotolerance response; however, hypocotyl growth was further enhanced when its application was continued during the heat-shock period. When the phthalimide treatment was given at 28°C, statistically similar increases were observed in respect of hypocotyl growth, but increase in root growth was less. The application of phthalimide to seedlings grown entirely at 28°C had a rather small growth-promoting effect.

Thus, a prior treatment of seedlings at an elevated temperature within a permissive range (40°C, 2 h) imparted protection to seedlings against heat-shock stress. Similar observations have been made by other workers[1,2]. The presence of phthalimide during the 40°C pretreatment and the heat-shock period enhanced the protective response, as has been observed[1] for GA_3. Hence AC 94,377 is capable of mimicking the heat-shock response induced by GA_3. In the present study, the phthalimide protection against heat-shock was provided by pretreatment not only at 40°C but also at the normal temperature of 28°C. In addition, both hypocotyl and root appear to be the site of the phthalimide effect, whereas in the case of GA_3 it is mainly the hypocotyl[1].

Application of phthalimide warrants further investigation to understand the mechanisms through which it triggers a protective response.

Thanks are due to Dr A. D. Pavlista of the American Cynamid Co. for a gift of AC 94,377.

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COLORIMETRIC METHOD FOR THE ESTIMATION OF *p*-COUMARIC ACID FROM THE BARK OF *OROXYLUM INDICUM* VENT

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p-COUMARIC acid has been estimated colorimetrically using ammonium vanadate–perchloric acid reagent[1], by reverse-phase high-pressure liquid chromatography[2], spectrophotometrically using 4-hydroxybenzaldehyde[3], and by high-pressure TLC with UV and fluorescence[4]. *p*-Coumaric acid from sunflower seeds was determined by titration with $KMnO_4^{(\mathrm{ref.}\ 5)}$. This method was rapid and simple, and gave good results.

p-Coumaric acid was extracted from the bark of *Oroxylum indicum* (Bignoniaceae) according to Subramaniyan and Nair[6]. It was further purified by preparative TLC using silica gel GF 254 as adsorbent and benzene : dioxane : acetic acid (90 : 25 : 4)[7] as solvent. The prominent spot obtained at R_f 0.45 was scraped out and the compound so obtained was recrystallized from methanol. The compound was confirmed as *p*-coumaric acid from its m.p., R_f and IR spectrum[8].

A more sensitive colorimetric method was established by reacting *p*-coumaric acid with molybdophosphoric acid in alkaline medium. A reference solution was prepared by dissolving 10 mg of *p*-coumaric acid in 100 ml of ethyl alcohol. To prepare a test solution bark extract[8] was concentrated, the residue so obtained was dissolved in 10 ml of ether, and 2 ml of this was made up to 100 ml with ethyl alcohol.

Three activated silica gel GF 254 plates were streaked with 0.25, 0.5 and 0.75 ml of reference and test solutions. Chromatography was performed using benzene:dioxane:acetic acid (90:25:4) as the solvent system. Scrapings of the band corresponding to *p*-coumaric acid were subsequently transferred to a test tube and extracted with 5 ml of ethyl alcohol. Following centrifugation the supernatant liquid was filtered through Whatman No. 42 filter paper into a 10-ml volumetric flask. To the above filtrate 3 ml of 5% molybdophosphoric acid and 1 ml of 1 N NaOH were added. The resultant blue chromogen was measured at 720 nm in a Carl–Zeiss speckol spectrocolorimeter. Percentage recovery of *p*-coumaric acid by this method was 98%. The *p*-coumaric acid content of the bark of *O. indicum* was found to be 1.84%.

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NEW HOST RECORDS OF THE BRUCHID *SULCOBRUCHUS KINGSOLVERI* ARORA AND THE PARASITE *OEDAULE STRINGIFRONS* WATERSON FROM INDIA

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DALBERGIA PANICULATA Roxb. (Leguminosae) is distributed throughout southern and central India, extending northwards to Oudh and the Siwaliks. The wood of this tree is used for building purposes and to make instruments. A small (200 g) sample of *D. paniculata* seeds was received at the Quarantine Laboratory of the Bureau from the National Botanical Garden, Lucknow. It was intended for export to the USA. On examination it was found that, about 2% of the seeds were damaged, and some live bruchids and parasites were recovered. These were later identified as *Sulcobruchus kingsolveri* Arora (Bruchidae: Coleoptera) and *Oedaule stringifrons* Waterson (Pteromalidae: Hymenoptera).

A study of the literature revealed that *D. paniculata* seeds are infested by *Bruchidius uberatus* (Fahraeus)[1]. There is no record of *S. kingsolveri* infesting *D. paniculata* seeds. However, *S. kingsolveri* is known to infest seeds of *Albizia* species[2]. The present record of *S. kingsolveri* infesting *D. paniculata* seeds is a new host record for this bruchid.

Oedaule stringifrons is a known parasite of the groundnut bruchid, *Caryedon serratus* (Olivier) (= *C. gonagra* (Fabricius)), and a reference to the published literature shows that *O. stringifrons* has never been detected as a parasite of *S. kingsolveri*. The present record of *O. stringifrons* along with *S. kingsolveri* from *D. paniculata* seeds constitutes a new host record for this parasite.

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APHIS GOSSYPII GLOVER, AN INSECT VECTOR OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) MOTTLE VIRUS—A NEW RECORD

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CARNATION is an ornamental plant; it is also used in the manufacture of sophisticated perfumes and medicines. Carnation mottle disease, caused by a virus, is characterized by chlorotic spots on young apical leaves, followed by mosaic chlorosis, mottling and streaks of yellowish-white colour on infected leaves. The infected plant shows stunted growth and bushy appearance. No insect vector has been reported so far for the carnation mottle virus (CMV). This communication reports an insect vector for CMV.

In nature insects are the main cause of the spread of plant viruses and there is a specific relationship between a virus and its vector[1].

Insects were collected from carnation fields and also from the neighbouring areas during morning, noon, evening and night hours. These were cultured in insect cages on healthy carnation plants. The insects were transferred directly from the infected carnation plant to healthy plants in one set and in another set a preliminary period of fasting was allowed before transmission experiments. During the fasting period the insects were placed in petri dishes covered with cellophane.

The infected plants on which insects were fed to acquire the virus were all of the same age. Plastic cages with nylon-covered windows were used for raising the stock culture and for all insect transmission trials. These cages were fixed over the young plants in 6-inch pots. To study the possibility of insect transmission of the virus three experiments, viz. preliminary screening of insects, virus–vector relationship and persistence of the virus in the vector, were conducted.

In the preliminary screening of insects, each insect species was given an acquisition feed for 1 h and then allowed a test feed of 1 and 12 h separately on healthy carnation plants. The results obtained with all the insect species except *Aphis gossypii* were negative. Only 10% transmission was recorded after 1 h test feed, and 100% after 12 h test feed.

To test virus–vector relationship five different acquisition feeds, each with a set of seven test feeds, were given to *A. gossypii*. For each combination of acquisition and test feed 10 healthy carnation plants were used, each with a batch of 10 *A. gossypii*. Vectors were transferred to healthy plants for different periods of test feed after subjecting them to 6 h fasting period before allowing the required acquisition feed (table 1). The results reveal that with increase of duration of acquisition and test feeds the number of infected plants increase regularly.

Table 1 *Effect of duration of acquisition and test feeds on transmission of CMV by Aphis gossypii*

	Number of plants infected out of 40 plants						
	Test feed (h)						
Acquisition feed (min)	1	2	4	6	8	12	24
10	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—
40	—	—	—	4	6	10	20
60	4	16	24	40	40	40	40
90	10	22	36	40	40	40	40

The persistence of virus in the vector was studied and the results revealed that the virus was retained within the vector for up to 8 days, indicating that the virus is of the persistent type. It is concluded that the vector can acquire sufficient virus in 60-min acquisition feed and 6-h test feed to give 100% transmission. Thus it is apparent that *A. gossypii* requires a latent period of 1 to 6 h to become infective.

2 July 1988; Revised 2 December 1988

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OCCURRENCE OF *BEAUVERIA BASSIANA* (BALSAMO) VUILL. ON SAPLING BORER *SAHYADRASSUS MALABARICUS* MOORE (LEPIDOPTERA: HEPALIDAE) IN KERALA, INDIA

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SAHYADRASSUS MALABARICUS Moore, popularly known as the sapling borer, is a polyphagous insect

Entomopathogen

pest that attacks saplings of a variety of forest trees. Its recorded hosts in Kerala include several species of forestry importance like eucalypts, *Tectona grandis* Linn. f., *Paraserianthes falcataria* (L.) Fosb., etc. The ecology, host range and possible control of this insect have been recently studied[1].

No information is available on the microbial pathogens associated with this insect and their possible role in control of the pest, probably because of its concealed mode of feeding. Instances of larval mortality due to fungal infection were noticed (figure 1) in some teak plantations in the Chalakudy Forest Division, Kerala, during September–October 1986 (temperature 28 ± 2°C, RH > 85%), when the infestation was prevalent on saplings along the boundaries, particularly when the weed growth was heavy. In such cases it was found to thrive well in plants like *Clerodendrum viscosum*, which also serves as an alternate host of this insect. Infected larvae appeared whitish because of profuse mycelial growth and were found underneath the frass covering the tunnel entry, usually with the anterior end protruding outside. The pathogen was later isolated and identified as *Beauveria bassiana* (Balsamo) Vuill. (IMI No. 313438). The pathogenicity was evaluated in the laboratory for assessing the efficacy of the fungus as a potential biocontrol agent against *S. malabaricus.*

The pathogen collected from affected *S. malabaricus* larvae was isolated on potato dextrose agar medium. For pathogenicity tests, a spore suspension prepared from a 7-day-old culture, with a concentration of 1.4×10^5 spores/ml adjusted by using a Neubauer haemocytometer, was sprayed on field-collected 3–4-month-old larvae maintained on an artificial diet. The experiment had three replicates, each with 10 larvae. Control larvae were sprayed with sterile water. The experiment was conducted at room temperature (28 ± 2°C and RH > 85%). Observations on the development of symptoms were made 24 h after inoculation and subsequently every 12 h till > 75% mortality occurred in treated larvae. Among the treated larvae, 80–90% died from fungal infection after 96 h; no mortality occurred in the control. The affected larvae were covered with a white mycelial growth on which profuse sporulation was noticed from 120 h onwards. From these results, *B. bassiana* appears to be a very effective pathogen of *S. malabaricus.*

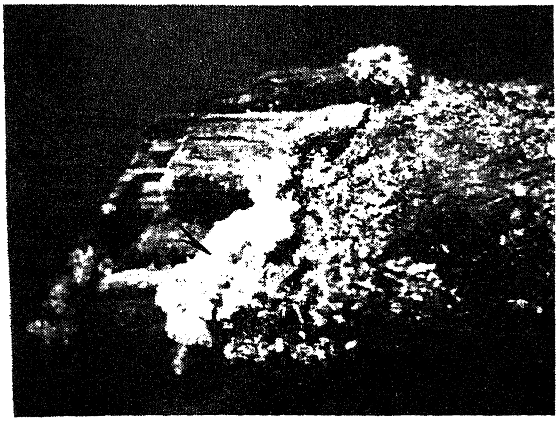

Figure 1. Infested sapling split open showing *B. bassiana*-affected *S. malabaricus* larva (arrow).

B. bassiana is well known as an entomophagous pathogen in several temperate and tropical countries[2–4]. It has a wide range of insect hosts and is considered to be a potential biocontrol agent[5].

In India, *B. bassiana* has already been reported to cause mortality of the teak skeletonizer, *Eutectona machaeralis* (Wlk.) in Karnataka[6]. It is for the first time that this pathogen is being reported on *S. malabaricus*, a stem-boring pest of several forest tree species.

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MATING DISRUPTION THROUGH BEHAVIOURAL MANIPULATION OF *SPODOPTERA LITURA* FABRICIUS

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SPODOPTERA LITURA Fabricius is a polyphagous pest on 112 species of plants belonging to 44 families. In India 40 species of cultivated plants and 24 wild plants have been reported as hosts. It is a regular major pest and often causes severe damage to cotton, tobacco, chilli, groundnut, castor, pulse crops, tomato, brinjal, cabbage, cauliflower, bhendi, sweet potato, banana and minor spice crops. Severe outbreaks of the pest were reported in cotton-growing tracts of the country in the past ten years.

Excessive dependence on insecticides against the pest leads to large-scale destruction of its natural enemies in the environment. The pest also defies the normal control interventions as it has developed resistance to DDT, BHC, endosulfan, carbaryl, malathion, fenitrothion, methylparathion and monocrotophos, and to *Bacillus thuringiensis* B[1–4]. Hence there is imminent need to develop and promote an ecologically suitable and environmentally compatible pest management strategy to tackle the pest effectively, and ultimately to prevent economic losses in crop production. Mating disruption through behavioural manipulation with the use of synthetic sex pheromones in an indigenous method using locally fabricated material has been investigated with a view to develop a viable strategy for the management of the pest.

Hollow fibres, plastic laminates and microcapsules were developed and used in mating disruption studies against *S. littoralis*, a close ally of *S. litura*, elsewhere. Efforts to obtain samples of these formulations for testing were not successful. This study was therefore designed and implemented using locally fabricated polythene vials as dispensers. The study was conducted on cultivators' holdings from the first week of December to the end of February for two successive years during 1984–85 and 1985–86.

Sixteen vials were impregnated with 1 g of the synthetic pheromone containing component 'A' (*Z*, *E*)-9,11-tetradecadienyl-1-ol acetate, and component 'B' (*Z*,*E*)-9–12-tetradecadienyl-1-ol acetate, in 10:1 ratio. Each vial was thus loaded with 6.25 mg of the pheromone. These 16 pheromone dispensers were set up equidistantly in a 0.4 ha chilli plot in two rows of 10 in the outer and 6 in the inner ring by hanging them from bamboo poles in such a way that the dispensers were 20 cm above the crop canopy. For comparison a control 0.4 ha plot of chilli crop was selected 1 km away from the experimental plot. At the central point of each plot a normal pheromone trap with a 2.5 mg pheromone dispenser and a dry polythene sleeve was set up as indicator trap to observe and record daily capture of *S. litura* male moths. The number of male moths trapped every day in the indicator traps and the number of egg masses laid were recorded to measure the impact of the pheromone on male orientation and mating disruption. The data obtained were subjected to *t* test for statistical significance.

Moth catches in indicator traps were very low in the experimental plot compared to the plot free from pheromonal influence in both years of the study (table 1). In the daily recording of the 13-week study, moths were not captured except on a few days (1 to 3 moths/trap/week) in the experimental plot. In the control, pheromone-free plots the moth catches were appreciably high, ranging from 5 to 115 and 11 to 6283 moths/trap/week during the first and second years of study respectively. The difference was significant, between experimental and control plots indicating the influence and impact of the pheromone permeated in the area on the orientation of male moths. Pest incidence in general was low during 1984–85; nevertheless both the seasons of the study showed identical trends. Field records of numbers egg masses also indicated sex disruption (table 1).

Campion[5] suggested the use of hollow fibres, plastic laminates and microcapsules for pheromone-mediated mating disruption in *S. littoralis*. In the present study the objective of permeating 1 g of pheromone in a 0.4 ha crop area was achieved with the use of locally available polythene vials set up at release points stationed at close spacing. Communication between male and female *S. litura* moths was effectively disrupted with this technique, which is thus an equally effective alternative to the slow-releasing imported formulations.

Assessment of the effectiveness of the pheromone and release method was based on the size of trap catches and egg mass counts, as has been done for *S. littoralis*[6, 7]. But Oyama[8] and Kobayashi *et al.*[9] have used tethered virgin females to provide a direct measure of mating levels in their studies with *S. litura*. They recommended the use of at least 15 traps/ha to achieve marked reduction in mating rate of teth

Table 1 *Catches of S. litura male moths and number of egg masses in pheromone-exposed and unexposed chilli plots*

Standard week	Unexposed plot: Mean no. of moths	Unexposed plot: Mean no. of egg masses	Pheromone-exposed plot: Mean no. of moths	Pheromone-exposed plot: Mean no. of egg masses
1984–85				
45	12 (4.46)	4 (3.00)	0 (1.00)	1 (2.00)
46	48 (7.92)	5 (3.23)	0 (1.00)	0 (1.00)
47	115 (11.72)	15 (4.87)	0 (2.41)	0 (1.00)
48	46 (7.78)	23 (5.79)	2 (1.00)	0 (1.00)
49	23 (5.79)	15 (4.87)	0 (1.00)	1 (2.00)
50	26 (6.09)	10 (4.16)	0 (2.00)	0 (1.00)
51	16 (5.00)	9 (4.00)	1 (2.41)	0 (1.00)
52	14 (4.74)	4 (3.00)	2 (1.00)	0 (1.00)
1	75 (9.66)	3 (2.73)	0 (1.00)	0 (1.00)
2	13 (4.60)	5 (3.23)	0 (1.00)	0 (1.00)
3	5 (3.33)	1 (2.00)	0 (1.00)	0 (1.00)
4	27 (6.19)	2 (2.41)	0 (1.00)	0 (1.00)
5	16 (5.00)	1 (2.00)	0 (1.00)	0 (1.00)
t	2.70*	22.19	2.70**	22.19**
1985–86				
45	293 (18.11)	30 (6.47)	0 (1.00)	4 (3.00)
46	238 (16.42)	180 (14.41)	2 (2.41)	5 (3.23)
47	211 (15.52)	195 (14.96)	1 (2.00)	0 (1.00)
48	47 (7.85)	108 (11.39)	0 (1.00)	0 (1.00)
49	460 (22.44)	10 (4.16)	0 (1.00)	0 (1.00)
50	379 (20.46)	261 (17.15)	3 (2.73)	0 (1.00)
51	6283 (30.26)	350 (19.70)	0 (1.00)	0 (1.00)
52	20 (5.47)	2562 (51.61)	1 (2.00)	4 (3.00)
1	1310 (37.19)	41 (7.40)	2 (2.41)	0 (1.00)
2	756 (28.49)	1060 (33.55)	1 (2.00)	1 (2.00)
3	295 (18.17)	365 (20.10)	0 (1.00)	0 (1.00)
4	68 (9.24)	140 (12.83)	2 (2.41)	1 (1.00)
5	11 (4.31)	18 (5.24)	0 (0.00)	1 (2.41)
t	3.50**	3.11**	3.50**	3.11**

Figures in parentheses are the $\sqrt{x+1}$ transformed values.

females. Kawasaki and Miyashita[10] achieved 50% mating suppression with a field application of 72 mg pheromone per 0.4 ha. Kehat *et al.*[11], Hall *et al.*[12] and Campion[5] achieved mating disruption in *S. littoralis* with Hercone dispensers at 1.5 g/0.4 ha, and with microcapsules at 5 to 10 g/ha and 100 g a.i./ha, as against 1 g pheromone used per 0.4 ha in the present study. The present results agree with those obtained by Mitchel and McLanghlin[13] with hollow fibres for *S. frugiperda*, and by Campion *et al.*[14] for *S. littoralis*.

27 June 1988

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BENEFICIAL EFFECTS OF ASPIRIN IN CYANATE-INDUCED HYPERCALCEMIA AND HYPERPHOSPHATEMIA IN RAT EYE LENS

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CYANATE reacts with lens protein and induces conformational changes[1]. Bendazac was found to decrease binding of cyanate to lens proteins and prevent cyanate-induced elevation of phase separation temperature in incubated rat lens[2]. Exposure to cyanate has been considered as an important risk factor in cataract formation in India[3] and England[4].

Investigations were carried out on the effect of cyanate and aspirin on the metabolism of calcium and phosphate in lens and serum of rat. These studies are of significance as both calcium and phosphate play key roles in a wide spectrum of biological processes including the formation of cataract.

Male albino rats were divided into two groups. Twenty-four rats of group I (control) were intraperitoneally injected with 0.1 ml Ringer's buffer solution, pH 8.2. Rats of group II (48) were injected with 0.1 ml cyanate (5 mg) dissolved in Ringer's buffer, pH 8.2. Twenty-four rats of group II were orally given 0.2 ml aspirin (9 mg in 60% alcohol) per day. All animals were sacrificed after 30 days and calcium (Ca^{2+}) and phosphate (HPO_4^{2-}) levels in blood and lens were determined by atomic absorption and spectrophotometric[5] methods respectively.

Table 1 shows that the blood urea level increased by about 52% in rats injected with cyanate. Feeding aspirin to cyanate-treated rats inhibited the increase in blood urea. The increased urea level in the cyanate-treated rats resulted in decrease of pH from 7.4 to 7.1. Reduced glutathione (GSH) and ascorbic acid levels in lens also decreased. Aspirin inhibited the decrease. The levels of calcium and phosphate in plasma increased by 45 and 10.6% respectively (table 2). Interestingly, the increase in plasma resulted in an increase in calcium and phosphate levels in lens by 53 and 33% respectively. The results also show that aspirin feeding produced significant inhibition of increase in calcium and phosphate levels, with complete inhibition in the case of lens.

Table 1 *Effects of cyanate and aspirin on blood pH, and urea, reduced glutathione (GSH) and ascorbic acid levels in blood and eye lens of rat*

	Blood		Lens	
	pH	Urea (mg/ 100 mg)	GSH (μg/g)	Ascorbic acid (μg/100 mg wet wt)
Control (*n* = 12)	7.4 ± 0.1	9.24 ± 1.32	1.40 ± 0.11	4.24 ± 0.24
Cyanate-treated (12)	7.1 ± 0.1	14.01 ± 1.42	1.06 ± 0.10	2.65 ± 0.19
Cyanate-treated, fed aspirin (12)	7.3 ± 0.1	10.6 ± 1.18	1.28 ± 0.12	3.62 ± 0.25

These studies show that cyanate induces uraemia and acidosis in blood, which are considered to be amongst the relevant factors involved in the processes leading towards development of diarrhoeal cataract[6]. The significant decrease in lens GSH and ascorbic acid is in agreement with earlier findings that their concentration decreased in almost all experimental cataracts and in human senile cataracts[7]. The cyanate-induced hypercalcemia and hyperphosphatemia in the lens also confirm results reported earlier[8]. The presence of aspirin possibly minimizes the charges on proteins and protein–water interaction, and thus prevents changes in the lens. The potential effect of aspirin on the lens depends on its concentration after systemic administration.

The authors are grateful to DST, New Delhi, for financial assistance. Thanks are due to Prof. J. J. Harding, University of Oxford, for a gift sample of cyanate.

Cataract

Table 2 *Effects of cyanate and aspirin on levels of calcium and phosphate in plasma and lens of rat*

	Plasma		Lens	
	Calcium (mg/100 ml)	Phosphate (mg/100 ml)	Calcium	Phosphate
Control ($n=12$)	10.27 ± 0.55	6.55 ± 0.31	0.60 ± 0.08	4.24 ± 0.21
Cyanate-treated (11)	14.95 ± 0.85	7.25 ± 0.29	0.92 ± 0.10	5.65 ± 0.24
Cyanate treated, fed aspirin (11)	8.01 ± 0.63	6.56 ± 0.71	0.66 ± 0.07	4.35 ± 0.20

23 August 1988; Revised 24 January 1989

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PRECIPITATION REACTIONS OF LECTINS WITH SERUM SAMPLES OF PATIENTS WITH VARIOUS MALIGNANT DISORDERS

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LECTINS are soluble proteins that can bind non-covalently to specific carbohydrate groups without modifying them chemically. By virtue of this property, lectins are useful in topographical studies of a wide variety of biological cell membranes. Their application to the study of tumour cells is a rapidly developing facet of cancer research[1–4]; they not only agglutinate but also transform cells. Lectins also precipitate certain proteins. In the present study an attempt has been made to distinguish between normal serum and serum of patients suffering from various malignant disorders by using different seed extracts. The seeds of many plants are known to contain lectins.

Extracts of 207 different seeds of plants belonging to 53 families were tested against serum samples of patients with history of various carcinomas or lymphoproliferative/haematologic disorders. Serum samples from normal healthy volunteer blood donors were used as controls. Seed extracts were prepared by a standard technique[5]. Each batch of lectin was standardized for protein concentration. Precipitation reactions were carried out by the Ouchterlony method[6]. The diffusion was carried out overnight at room temperature in a wet chamber.

Table 1 shows specific and non-specific precipitation activities in 26 seed extracts with serum samples of normal individuals and patients suffering from various carcinomas. It is interesting that normal serum samples vary from serum samples derived from various carcinoma patients. Seed extracts 1–4 gave non-specific precipitin bands; extracts 5 and 6 differentiated serum of patients with carcinoma of rectum from serum of normal individuals and patients with other malignant disorders; extracts 7–16 gave specific precipitation bands with serum from patients with carcinoma of gall bladder; 17 and 18 gave precipitin bands with serum of patients with carcinoma of stomach; 19 gave positive reaction with serum from patients suffering from malignancies of the ovary; 20 and 21 gave precipitation reaction with the serum of breast carcinoma patients; extracts 22–26 gave precipitin bands with serum from patients with carcinoma of scalp. No precipitation reaction was observed with serum from patients with carcinoma of larynx or broncheogenic carcinoma.

From the above results it is clear that serum of normal individuals can be differentiated from that of patients with various malignant disorders on the

Table 1 *Precipitation reactions of lectins with serum from normal individuals and patients with various carcinomas*

Sl. no.	Plant	Carcinoma of rectum (*n* = 5)	Carcinoma of gall bladder (*n* = 5)	Carcinoma of stomach (*n* = 5)	Malignancy of ovary (*n* = 5)	Carcinoma of breast (*n* = 5)	Carcinoma of scalp (*n* = 5)	Carcinoma of larynx (*n* = 5)	Broncheogenic carcinoma (*n* = 5)	Normal (*n* = 80)
Non-specific precipitins										
1.	*Queracus incana*	5	5	5	5	5	5	5	5	80
2.	*Tamarindus indica*	5	5	5	5	5	5	5	5	20
3.	*Terminalia chebula*	5	4	3	3	2	3	5	5	30
4.	*Ipomia pinnata*	5	3	3	2	1	4	5	3	45
Specific positive precipitins										
5.	*Bauhinia purpurea*	5	—	—	—	—	—	—	—	—
6.	*Eucalyptus resnifera*	5	—	—	—	—	—	—	—	—
7.	*Ipomea palmata*	—	5	—	—	—	—	—	—	—
8.	*Cicer arietinum* (Kabuli)	—	5	—	—	—	—	—	—	—
9.	*Medicago falcata*	—	4	—	—	—	—	—	—	—
10.	*Aesculus indica*	—	5	—	—	—	—	—	—	—
11.	*Albizzia lucida*	—	3	—	—	—	—	—	—	—
12.	*Derris robusta*	—	3	—	—	—	—	—	—	—
13.	*Putranjiva roxburghii*	—	5	—	—	—	—	—	—	—
14.	*Centaurea moschata*	—	5	—	—	—	—	—	—	—
15.	*Gossypium indicum* (G-27)	—	3	—	—	—	—	—	—	—
16.	*Cicer arietinum* (F-378)	—	4	—	—	—	—	—	—	—
17.	*Acacia catechu*	—	—	5	—	—	—	—	—	—
18.	*Acacia tortalis*	—	—	5	—	—	—	—	—	—
19.	*Albizzia amara*	—	—	—	5	—	—	—	—	—
20.	*Parkinsonia aculeata*	—	—	—	—	5	—	—	—	—
21.	*Trewia nudiflora*	—	—	—	—	5	—	—	—	—
22.	*Dalbergia sissoo*	—	—	—	—	—	5	—	—	—
23.	*Lagerstroemia flosreginae*	—	—	—	—	—	3	—	—	—
24.	*Strychnos nuxvomica*	—	—	—	—	—	5	—	—	—
25.	*Prosopis cineraria*	—	—	—	—	—	4	—	—	—
26.	*Acacia dealbata*	—	—	—	—	—	4	—	—	—

basis of a simple precipitation test. Our results highlight the use of lectins as a diagnostic tool for neoplastic diseases. It is possible that some of these specific +ve precipitating lectins may be of further help in detecting changes in serum proteins at early stages of malignancy even before the disease is clinically manifested.

9 August 1988; Revised 10 November 1988

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BOOK REVIEW

Annual Review of Computer Science, vol. 3, 1988, pp. 420, (eds.) J. F. Traub, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, CA 94303, USA), Price: US$ 45, elsewhere $ 49.

In this volume there are 13 interesting articles surveying or summarizing the recent advances in several areas of computer science. The fields of artificial intelligence and computer science theory claim four articles each. Three articles are devoted to software science and two articles are devoted to special topics.

A successful application of AI techniques is in the development of expert systems software. In the review 'Fundamentals of expert systems' the authors discuss the characteristics of expert systems and their dependence on the extent of the knowledge and database of specific problem areas. The article 'Image analysis' deals with the problem of defining the relationship between a real object and its two-dimensional digitized image on the screen. The related algorithmic techniques are reviewed. In the article 'Resolution theorem proving', the methods of automated deduction are applied to propositional calculus and equality reasoning. In the review 'Commonsense physics' the author describes the new subfield of AI called qualitative physics. This is concerned with modelling expert reasoning as well as common sense and also with uncovering the ties between qualitative and traditional models of physics.

Of the four articles on computer science theory, 'Parallel algorithmic techniques for combinatorial computation' is of interest to those dealing with software development for parallel processors sharing a common memory. The recent algebraic approaches to algorithmic solutions of geometric reasoning problems are surveyed in 'Algebraic methods for geometric reasoning'. Another related article, 'Computational geometry', reviews some of the foundations of the subject—convex hulls, Voronoi diagrams and arrangements. Its author also discusses some of the exciting developments in this area discovered by Guibas *et al.* The computational cost of solving problems with an algebraic flavour is the subject of the review 'Algebraic complexity theory'. In it the effect of well-designed algorithms on the upper bound of complexity and the effect of intrinsic properties of problems on the lower bounds of complexity are nicely discussed in a question–answer format.

Among the three articles on software science, the most interesting is a review of the Lisp language. In it the power, simplicity and vitality of Lisp, responsible for its continuing popularity, are discussed. The second article discusses the development, during the past 25 years, of specific principles for reasoning about the correctness of programs for solving specific problems. Attention is given to deterministic sequential programs and their logical foundations. The third article discusses techniques of ensuring the secrecy, integrity and accessibility of data stored in a database.

One of the two special articles in this volume is on computational approaches to an exponential-time problem in protein folding. The problem of spontaneous folding of protein molecules into unique three-dimensional structures is well explained for computer scientists. The tremendous progress made since G. N. Ramachandran *et al.* (1963) pioneered the first conformational energy estimates on dipeptides is also reviewed. Finally there is a review on the importance of product design, process design, facilities design and activity planning in industrial manufacturing based on computers.

To sum up, this volume must find a place in computer science libraries everywhere.

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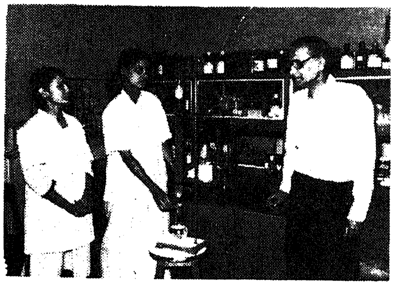
THE SCIENTIFIC COMMUNITY

We intend to start a regular feature on news about Indian scientists where we hope to publish short notices of awards and honours, appointments, transfers, retirements and obituaries. We shall also publish notices of election of Fellows and Associates to our Academies. Contributions to these columns are invited for consideration for publication.

—Editor

Awards and honours



The Liquid Crystal Laboratory of the Raman Research Institute, Bangalore, headed by Prof. S. Chandrasekhar, was among the 'most distinguished universities and scientific institutions in the world' honoured by the University of Bologna, Italy, on 10 June 1989, on the occasion of its 900th anniversary celebration.

Prof. Roddam Narasimha, Director, National Aeronautical Laboratory, Bangalore, has been elected (13 February 1989) Foreign Associate of the National Academy of Engineering, USA. It is stated that Dr Narasimha's election to the Academy 'signifies recognition of his distinguished contributions to the broad fields of engineering, engineering science and technology, and to the world in which we live'.

Prof. P. R. Pisharoty, Professor Emeritus, Physical Research Laboratory, Ahmedabad, is to receive the International Meteorological Organization Prize. The announcement by the World Meteorological Organization, Geneva, states, 'Throughout his long and active career, Prof. Pisharoty has promoted the science of meteorology, particularly in furthering studies on monsoons and in the field of remote sensing, to the continuing benefit of the global community'.

Prof. C. N. R. Rao, Director, Indian Institute of Science, Bangalore, was awarded the Hevrovsky Gold Medal by the Czechoslovakian Academy of Sciences, on 29 May 1989. The medal was for the monumental contributions of Prof. Rao to chemical sciences, especially for research contributions to spectroscopy and solid state chemistry. The recent work of his group on superconductors was also highlighted.

Prof. Rao has also been elected Foreign Member of the USSR Academy of Sciences.

Dr P. K. Ray, Director, Industrial Toxicology Research Centre, Lucknow, has been elected Member of the New York Academy of Sciences. The election is in recognition of his contributions in immunotoxicology and cancer.

Dr R. K. Bhandari, Director, Central Building Research Institute, Roorkee, was awarded the Om Prakash Bhasin Award for 1988 in the field of engineering and transport on 30 March 1989. Dr Bhandari is also Vice-President (1989–92) of the International Council for Building Research Studies and Documentation.

Dr M. R. Das, Centre for Cellular and Molecular Biology, Hyderabad, and Prof. T. Ramasarma, Department of Biochemistry, Indian Institute of Science, Bangalore, have been given the FICCI Award in life sciences for 1988.

Prof. D. Guptasarma, National Geophysical Research Institute, Hyderabad, has been selected as one of the recipients of the National Mineral Award for 1986–87.

Dr A. V. Rama Rao, Director, Indian Institute of Chemical Technology, Hyderabad, has been conferred the Ranbaxy Research Award for 1987 in pharmaceutical sciences.

Indian Academy of Sciences—Associates 1989

Mr G. Athithan, Advanced Numerical Research and Analysis Group (ANURAG), DRDO, Hyderabad—Computer software

Dr B. Basu, Tata Research Development Design Centre, Pune—Thermal science and engineering

Dr S. K. Chakrabarti, Tata Institute of Fundamental Research, Bombay—Theoretical astrophysics

Dr Rukhsana Chowdhury, Indian Institute of Chemical Biology, Calcutta—Molecular biology

Dr A. A. Deshpande, Raman Research Institute, Bangalore—Observational radioastronomy

Dr G. Ganguly, Indian Association for the Cultivation of Science, Calcutta—Optoelectronic materials

Dr U. Maitra, Indian Institute of Science, Bangalore—Organic reactions

Dr A. Mehra, University of Bombay, Bombay—Chemical reaction engineering

Dr S. Mohanty, Indian School of Mines, Dhanbad—Structural geology

Dr N. Nitsure, Tata Institute of Fundamental Research, Bombay—Algebraic geometry.

INSA Young Scientist Medal 1989 Awardees

Dr Joyoti Basu, Research Associate (CSIR), Department of Chemistry, Bose Institute, Calcutta—for characterization of yeast lectin and spectrin aggregation in erythrocytes.

Dr Bhabatosh Chanda, Computer System Engineer, Electronics and Communication Sciences Unit, Indian Statistical Institute, Calcutta—for development of mathematical models and methods for image processing and pattern recognition.

Dr V. Chandrasekhar, Assistant Professor, Department of Chemistry, Indian Institute of Technology, Kanpur—for contributions towards the synthesis of novel phosphorus ring-compounds and their reactions.

Dr Debashish Chowdhury, Assistant Professor, School of Physical Sciences, Jawaharlal Nehru University, New Delhi—for work on the theory of spin glasses.

Dr V. K. Dadhwal, Scientist 'SD', Land Resources Division, RSA Space Applications Centre, Ahmedabad—for work on modelling the effect of weather on growth and yield of wheat, and the application of satellite data and computer-based techniques for crop inventory.

Dr Deepti Deobagkar, Lecturer, Department of Zoology, University of Poona, Pune—for a sensitive assay for the detection of 5-methylcytosine in DNA and its application in studies of X-chromosome inactivation.

Dr Gautam Kumar Dey, Scientific Officer (E), Physical Metallurgy Division, Bhabha Atomic Research Centre, Bombay—for contributions to the production, detailed characterization and property evaluation of reactive metal–metal glasses, quasi-crystalline phases and high-temperature alloys.

Dr Madhu Dikshit, Scientist 'B', Pharmacology Division, Central Drug Research Institute, Lucknow—for work on the identification of a new platelet pro-aggregatory protein factor in thrombotic mouse plasma.

Dr Biswapati Mandal, Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Kalyani—for work on chemical transformation of applied zinc under submerged soil conditions.

Dr Anurag Mehra, Lecturer in Chemical Engineering, Department of Chemical Technology, University of Bombay, Bombay—for the formulation of a unified, comprehensive theory of microphase catalysis applicable to a variety of gas–liquid, liquid–liquid and solid–liquid reactions, and for the experimental verification of their salient features.

Dr Anand Mohan, Lecturer in Metamorphic Petrology, Department of Geology, Banaras Hindu University, Varanasi—for work on pressure–temperature characterization of granulites of South India.

Dr S. Mohanty, Lecturer, Department of Applied Geology, Indian School of Mines, Dhanbad—for structural studies related to unravelling basement-cover relationships in Rajasthan.

Dr Nitin Nitsure, Research Associate, School of Mathematics, Tata Institute of Fundamental Research, Bombay—for contributions to the study of moduli of vector bundles and the computation of their cohomology.

Dr Kanchan Pande, Post-Doctoral Fellow, Physical Research Laboratory, Ahmedabad—for work on characterization of ultramafic nodules in Deccan basalts.

Dr N. Ramesh, Research Associate, Molecular Biophysics Unit, Indian Institute of Science, Bangalore—for an innovative approach to the role of Z-DNA structure in gene expression.

Dr Debraj Ray, Professor, Indian Statistical Institute, New Delhi—for mathematical contributions to the theory of games and economic theory.

Dr Daman Saluja, Research Scientist 'A', Department of Botany, University of Delhi, Delhi—for contributions on reversible phosphorylation of ribulose triphosphate carboxylase and its activation.

Dr N. Viswanathan, Research Associate, Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai—for work on the circadian rhythm in the locomotor activity of the Indian pygmy field mouse.

Appointment

Dr G. P. Phondke has been appointed Director of Publications & Information Directorate, CSIR New Delhi, with effect from 26 June 1989. Dr Phondke joined the Biomedical Group of the Bhabha Atomic Research Centre, Bombay, in 1961 and worked in the areas of immunology, cell surface biophysics, radiation biology and cancer. From 1983 to 1986 Dr Phondke edited *Science Today.* He then became science editor for daily newspapers in the Times of India Group of Publications.

Retirement

Professor G. V. Anantha Iyer, Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, retired on 31 July 1989. Prof. Anantha Iyer got his B.Sc. degree from the University of Mysore in 1948, and the master's degree from Central College, Bangalore, in 1960. He joined the Department of Inorganic and Physical Chemistry, IISc, as a Research Assistant in 1962, and obtained the Ph.D. degree for his investigations on 'Geochemistry of the alkali feldspars and origin of gold–quartz veins in the Precambrian of Mysore'.

Prof. Anantha Iyer is a rare and, as far as this country is concerned, unique example of an outstanding geologist working in tandem with chemists to unravel the mysteries of rocks and minerals. He was instrumental in introducing courses in crystal chemistry in the IPC Department way back in the sixties, and has also taught engineering geology to M.E. students in civil engineering and mineralogy to B.E. and M.E. students in metallurgy.

Prof. Anantha Iyer has made outstanding contributions to geochemistry and mineralogy. The work of Prof. Anantha Iyer and his coworkers has led to a better understanding of the crustal evolution of the Karnataka (Dharwar) Craton and the spatially aligned gneiss–granulite region of Tamil Nadu and Kerala. Notable among his contributions to mineralogy are studies of the structural state of plagioclases, anthophylites, cordierites and chloritoid minerals, in particular the order–disorder phenomenon in chloritoids. He has also carried out several significant physicochemical investigations on economically important natural deposits such as asbestos, magnesite, gold and copper ore bodies located in Karnataka, Andhra Pradesh and Tamil Nadu.

Prof. Anantha Iyer is a Fellow of the Geological Society of India. He is also Member of the Indian Earth Sciences Association, and Member, Karnataka Association for the Advancement of Science. He has also been a member of the Working Group of the National Coordinated Project on 'Experimental petrology and high-pressure and temperature studies' set up by the Department of Science and Technology.

On the occasion of the retirement of Prof. Anantha Iyer, a symposium on geochemistry, chemistry and physics of minerals was held in the IPC Department, IISc. Twelve papers in civil engineering, metallurgy, geology, geochemistry, physics, materials science and chemistry were presented, emphasizing the truly multidisciplinary character of geochemistry. This issue of Current Science also contains a paper by G. V. Anantha Iyer and S. N. Gananath (EPR spectroscopic studies of calcic-plagioclases from the Archaean anorthosites of Holenarasipur, Karnataka Craton, South India, pp. 915–917).

—Editor

Obituaries

S. V. Anantakrishnan (*b*. 4.10.1908, *d*. 17.1.1989) was Professor and Head, Department of Chemistry, Madras Christian College, Madras. He was distinguished for important contributions to the application of principles of physical chemistry to organic chemistry.

Dr C. R. Dasgupta (*b*. 16.10.1899, *d*. 3.2.1989) was Head, Department of Haematology, and Deputy Director, School of Tropical Medicine, Calcutta. He worked on various haematological problems and on kala-azar.

C. G. Khatri (*b*. 4.8.1931, *d*. 31.3.1989) was Professor of Statistics, Gujarat University, Ahmedabad. His work included matrix algebra, multivariate distribution theory, characterization of distributions, and optimality of certain functions of matrix arguments.

S. G. Srikantia (*b*. 9.9.1926, *d*. 6.5.1989) was Director, National Institute of Nutrition, Hyderabad.

NEWS

Science journalism to get impetus

The Indian Science Writers Association (ISWA) in New Delhi is now putting more into the major exercise of increasing the number, visibility and impact of science reports in the country's newspapers. While science reporting is now finding more acceptance, it is not yet recognized as an important component of the newspapers. ISWA hopes that its new science feature service will help improve the situation considerably.

Last month ISWA organized workshops on advanced techniques in science communication at New Delhi and Bangalore. Participants included freelance science writers, science reporters and science editors. The workshops were conducted by Prof. A. J. Meadows of Loughborough University of Technology, UK, and Mr Martin Redfern, a producer in the Science Unit of the BBC. There were several exercises for the participants, including article content and readability analysis, and interviewing and writing. The problems of translation of scientific terminology, the sources of material for science journalists, the role of science fiction in fashioning the popular image of science, and the use of radio in science communication were also discussed. The workshops were part of ISWA's efforts at improving science journalism in the country.

Meanwhile, the Joint Media Committee of the Indo-US Subcommission on Education and Culture decided at its last biannual meeting that the United States will help train Indian television producers, directors and editors in science film-making. One of the planned projects under this arrangement is a series of workshops on production of science programmes for television.

The public understanding of science is being increasingly debated, and serious efforts to improve it are becoming important both for the public on the one hand and for the scientific community and science policy-makers on the other.

Journal costs cause worry

The rapidly increasing prices of scientific journals are posing serious problems to libraries and scientists. An article in a recent issue (24 July) of *The Scientist* (published by the Institute for Scientific Information, Philadelphia, USA) discussed the ramifications of the problem. While the soaring subscription costs are forcing (American) libraries to cancel subscriptions and delay orders of new journals, scientists who depend on these journals are increasingly worried about decreased access to a wide range of information and to the latest reports.

The Association of Research Libraries (ARL), which represents 119 US and Canadian libraries, conducted two studies of journal prices last year. As a solution to the problem of libraries' inability to meet the growing costs, ARL recommended that scientists should consider submitting their papers to non-profit rather than to for-profit journals and that granting agencies could also identify preferred, non-profit journals for reporting research results. But Eugene Garfield, editor-in-chief of *The Scientist*, points out that this recommendation has no supporting data that would allow a fair comparison between non-profit and for-profit journals. He recommends a comprehensive audit of all scientific publishers that would (i) help libraries make better-informed decisions about journal selection, (ii) be useful to authors in selecting the most efficient and responsive journals, and (iii) benefit publishers by pointing out deficiencies.

While Indian scientific journals are not prohibitively expensive, the Indian scientific community also faces the problem of non-availability of much-needed foreign and international journals because of the high cost. University and research institute libraries have a considerable problem in trying to satisfy several faculty committees representing different subjects. In the exercise of managing costs, the demands of scientists in the 'fashionable' areas may squeeze the even modest requirements of other areas.

Scientific publishing is such an essential component of science that solutions to these problems will have to be found soon.

CORRESPONDENCE

Readers may have noticed that we now have a correspondence column. Letters (less than 500 words) on matters concerned with science are invited for consideration for publication. We also intend to publish correspondence on scientific matters, not limited to those arising from an article published in Current Science.

—Editor

Premature failure in Ni–Cd batteries

Large numbers of rechargeable sealed nickel–cadmium batteries are in use all over the world. The rechargeable cells cost almost ten times as much as dry cells but are cheaper in the long run: even with a minimum of 20 recharges, the extra cost is recovered. Usually one can expect 50 or more recharges, although the capacity gradually decreases until it becomes uneconomical (usually after 100 recharges). In a well-sealed cell this loss of capacity is slow. I have some cells bought twenty years ago and used heavily, but still serviceable.

Unfortunately the ordinary user complains frequently of cells failing early. One common cause is leakage of electrolyte due to poor sealing. But frequently there is failure for no apparent reason. The cell shows zero volts and fails to charge. I tried heating to revive one such cell but to no effect. But when ten times the recommended charging current (equal to one hour charge rate) was passed, the cell regained charge. The dead cell usually shows full 1.25 volts after a minute or two of high-density charge. The full capacity is regained after putting it on normal charge.

After a study of several cells during the last fourteen years I found that such failures tend to occur towards the end of a deep discharge. Resistance measurement shows that the cell electrodes are shorted. One possible reason is the growth of dendrites or whiskers of cadmium metal crystals resulting in bridging of the electrodes. I think the large charging current melts the thin dendrite and removes the short. Cutting open such shorted cells and examining the electrode surface with a microscope may confirm this.

Some manufacturers are reported to be incorporating dendrite-inhibiting chemicals. But I have found premature failure in almost all makes and sizes of cells. Out of nearly 100 cells I have used, of various makes, about 60 failed and were revived, some two or three times during their life. Ultimately such cells fail to reactivate. I am writing this hoping that some research laboratory would take up the idea.

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A comment on the above:

The failure process of a sealed Ni–Cd battery is often a complex sequence of chemical and physical changes in the positive and negative electrodes, electrolyte or separator. Under normal conditions of design, fabrication and operation, sealed cells give 1000–2000 cycles (10–20 years of calendar life) in the wet condition, with deep discharge between the cycles. Unexpected failures can arise from inappropriate charging conditions (temperature, rate, amount of overcharge and mode of charging), cell reversal, or development of internal shorts. Irreversible failures can arise from either internal short or loss of electrolyte only.

For a sealed cell, the use of ten times the normal charging rate is always *dangerous* for the following reasons:

(i) Since the state of charge of the electrodes is not known, there is a possibility that all the current is used for gas evolution, causing pressure build-up, and, eventually, explosion due to gas generation in excess of the recombination rate (especially if the safety valve is not working or is absent).

(ii) Since the internal resistance of the cell is very high (as a result of failure in some cases) the Joule heating (I^2R) can increase the temperature of the cell, and this is catastrophic in combination with (i).

(iii) If the failure is due to electrolyte starvation, charging at higher rate can only worsen the condition.

(iv) Most of today's high-quality sealed Ni–Cd cells have ceramic-to-metal or combination glass/ceramic-to-metal seals. They work for more than ten years under normal charging conditions but can fail owing to stress-cracking under over-pressure.

In fact, for most of these cells, users are warned never to exceed the normally recommended charging rate for fear of explosion. There are separate designs for fast charging (3 *C* to 5 *C*) and superfast charging (5 *C* to 20 *C*) of sealed Ni–Cd cells, with devices to monitor pressure and temperature.

The failure of cadmium electrodes due to dendrite growth is only one reason for shorting and this problem has been successfully solved by employing several additives.

Articles that deal with failure modes of sealed Ni–Cd cells:

1. Gross, S., *Energy Convers.*, 1971, **11**, 39.
2. Durgaprasad, M., *Application of electrochemical kinetics to predict the failure modes of Ni–Cd cells*, Ph.D. thesis, Indian Institute of Science, Bangalore, 1984.
3. Salkind, A. J. and Duddy, J. C., *J. Electrochem. Soc.*, 1962, **109**, 360.

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The Battery Society of India has announced a 68-page publication from the Cadmium Association which contains the texts (and brief reports on discussions) of the thirteen papers presented at a seminar in Paris in April 1988. The seminar reviewed the features of performance of the main types of Ni–Cd batteries, currently available; applications in industry, railways, aircraft and automatic guided vehicles; photovoltaic applications; emergency lighting; small consumer batteries and battery-powered equipment; research and technological developments; and recycling. The publication is available (Rs. 300) from The Battery Society of India, No. 7 Shopping Centre, Block B-6, Safdarjung Enclave, New Delhi 110 029.

—Editor

Publishing codes

This has reference to the article 'A rapid technique for obtaining leaf prints for stomatal count with Fevicol' (*Curr. Sci.* **58**, 640–641, 1989) by K. A. Nayeem and D. G. Dalvi. I would like to bring it to your notice that imprinting by Fevicol was published from my research laboratory in 1969. I am citing the complete reference list of our papers on such techniques.

1. Inamdar, J. A. and Patel, R. C., *J. Microsc.*, 1969, **90**, 269.
2. Inamdar, J. A., Patel, R. C. and Bhatt, D. C., *Z. Wiss. Mikrosk. & Mikrosk. Tech.*, 1970, **70**, 140.
3. Bhat, R. B., Gangadhara, M., Bhatt, D. C. and Inamdar, J. A., *Curr. Sci.*, 1976, **45**, 344.
4. Inamdar, J. A., Gangadhara, M. and Bhat, R. B., *Microsc. Acta*, 1976, **78**, 39.

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We recognize the seriousness of the problem pointed out in this letter. We are tightening editorial procedures and also requesting reviewers to guard against publication of reports purporting to describe new work but actually only rehashing old ideas. Unfortunately in certain cases it seems it is rather difficult to detect such instances. One hopes authors themselves become more responsible.

—Editor

Dr S. Kanjlal, Department of Geology, Banaras Hindu University, Varanasi, has asked for details of S&T cooperation between India and Japan. The information may be obtained from the Indo–Japan Study Committee, Planning Commission, Yojana Bhavan, New Delhi 110 001.

—Editor

COMMENT

PROTEIN CRYSTALLOGRAPHY AND THE STRUCTURE OF INSULIN*

THE origin of protein crystallography can be traced to the first recording of the diffraction pattern from crystals of the protein pepsin in 1934 by J. D. Bernal and Dorothy Crowfoot (Hodgkin)[1]. This was soon followed, in 1935, by the X-ray diffraction photography of crystals of the well-known protein hormone insulin by Dorothy Crowfoot Hodgkin[2]. Professor Hodgkin often describes the X-ray photography of insulin (which were later identified as those of 2Zn insulin with two zinc ions per insulin hexamer) as the most exciting event in her long and illustrious scientific career, matched only by the eventual solution of its structure at 2.8 Å resolution 34 years later[3].

I remember how, at the International Congress of Crystallography at Stony Brook, NY (in 1969), Dorothy Hodgkin stole the thunder when it was announced that the structure of insulin had been solved in her laboratory. It is also characteristic of her that the paper was presented by the youngest member of her group. It is a reflection of her steadfastness and singlemindedness in pursuing a difficult problem for long years that no one in the insulin group of her laboratory in 1969—Guy Dodson, Eleanor Dodson, Tom Blundell and M. Vijayan—who finally helped her accomplish the solution of the structure, was even born when she started her work on insulin in 1935! It must be a matter of great happiness to her that these 'young crystallographers' have established renowned schools, at York, London and Bangalore, respectively. Subsequent to the solution of the structure, Hodgkin and her colleagues concentrated on its refinement at high (1.5 Å) resolution. The refined structure has been presented at length in the 6 July 1988 issue of the *Philosophical Transactions of the Royal Society, London*[4]. The paper contains the most comprehensive and elaborate discussion to date on a protein structure and the associated water molecules.

Insulin has been involved in many landmarks in protein biochemistry. It was the first protein to be sequenced and also the first to be chemically synthesized. More recently, the insulin gene has been among the first to be cloned using genetic engineering techniques and the protein has been expressed in large quantities. Its role in protein crystallography has been no less important. As indicated earlier, crystals of insulin were among the first protein crystals to be X-ray-photographed. Among the early applications of the then recently developed Patterson method in the early thirties was the one on insulin crystals[5], a formidable undertaking indeed in the pre-computer days. Rotation and translation functions are the most important components of the now widely used molecular replacement method. The elucidation of the internal symmetry in insulin crystals was among the earliest applications of these functions[6]. The comparatively small size of insulin molecules and the tight packing of molecules in the crystals made the preparation of several independent heavy-atom derivatives rather difficult. The derivatives ultimately used in the structure solution were related to one another to varying degrees. This, as well as the total absence of centric reflections in the data, led to serious crystallographic problems. These were overcome largely through the extensive use of methods based on X-ray anomalous scattering[7,8]. The experience gained through the X-ray analysis of 2Z insulin in the effective use of anomalous scattering in protein crystallography has indeed been of considerable general interest. 2Zn Insulin has also been among the first protein crystal structures to be refined at high resolution. The work on insulin led to valuable contributions toward the development of protein refinement methodologies and the elucidation of the nature of problems encountered during refinement[9-11].

The detailed paper on 2Zn insulin in the *Philos. Trans.* indeed marks the culmination of an effort of epic proportions spread well over half a century. During this period, Professor Dorothy Hodgkin determined the structures of several important molecules, including those of cholesterol, penicillin and vitamin B_{12}, came to be recognized as perhaps the most distinguished X-ray crystallographer in the world, and received many honours, including the Nobel Prize. However, insulin has been the molecule

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*On receiving the paper 'The structure of 2Zn pig insulin crystals at 1.5 Å resolution'; *Philos. Trans. R. Soc.* (*London*), 1988, **B319**, 369–456.

closest to her heart and the work on it spanned her entire scientific career. In this process she trained generations of crystallographers in different continents and laid the foundations for the spread of protein crystallography to different parts of the world. The *Philos. Trans.* paper itself bears testimony to this truly international character of hers. The authors of the paper belong to five different countries, namely England, Australia, New Zealand, Japan and India.

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1. Bernal, J. D. and Crowfoot, D., *Nature* (*London*), 1934, **133,** 794.
2. Crowfoot, D., *Nature* (*London*), 1935, **135,** 591.
3. Adams, M. J., Blundell, T. L., Dodson, E. J., Dodson, G. G., Vijayan, M., Baker, E. N., Harding, M. M., Hodgkin, D. C., Rimmer, B. and Sheet, S., *Nature* (*London*), 1969, **224,** 491.
4. Baker, E. N., Blundell, T. L., Cutfield, J. F., Cutfield, S. M., Dodson, E. J., Dodson, G. G., Hodgkin, D. M. C., Hubbard, R. E., Isaacs, N. W., Reynolds, C. D., Sakabe, K., Sakabe, N. and Vijayan, M., *Philos. Trans. R. Soc.* (*London*), 1988, **319,** 369.
5. Crowfoot, D., *Proc. R. Soc.* (*London*), 1938, **A164,** 580.
6. Dodson, E., Harding, M. M., Hodgkin, D. C. and Rossman, M. G., *J. Mol. Biol.*, 1966, **16,** 227.
7. Dodson, E. and Vijayan, M., *Acta Crystallogr.*, 1971, **B27,** 2402.
8. Vijayan, M., *Structural Studies on Molecules of Biological Interest*, (eds) G. Dodson, J. P. Glusker and D. Sayre, Clarendon Press, Oxford, 1981, pp. 260.
9. Isaacs, N. W. and Agarwal, R. C., *Acta Crystallogr.*, 1978, **A34,** 782.
10. Vijayan, M., *Acta Crystallogr.*, 1980, **A36,** 295.
11. Dodson, G. G., *Refinement of Protein Structures, Proceedings of the Daresbury Study Weekend*, (eds) P. Machin, J. Campbell and M. Elder, SERC Daresbury, 1981, pp. 95.

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POLICY ON INDIAN EDITIONS OF FOREIGN S&T PERIODICALS

The Department of Science and Technology (DST) is reconsidering the national policy regarding the publication of editions of foreign science and technology periodicals and magazines in India. This question has parallels in the problem India faced in building up its technology. To achieve self-reliance the policy pursued was: limit the imports of technology; raise customs barriers to protect our products; encourage developmental activities in Indian laboratories; etc. The policy had its successes but some persons were not sure whether any real progress was made. For example: with the passage of time, old, decrepit industrial processes continued to exist, defended by vested interests; industries did not form R&D groups even to absorb the imported know-how; no attempt was made to improve the technology or the products; nor was there any effective interaction between industries and Government-funded laboratories. So a decision was taken to permit imports of products and technology that would encourage a conscious improvement of quality so that our products could compete in the world market. The effect of this experiment is still to be properly assessed. (This is a good theme for later discussion in these columns.)

Following a similar path a decision was taken by the Government not to allow foreign science and technology journals to be published in India. More than thirty years have passed since then and so the Government wishes to examine whether a revision of policy is necessary. If foreign journals are published in India, there may be some saving of foreign exchange for us as we get many journals from abroad. But these journals will be published in India only if there is a market, which means profits to the publisher. More important, however, is the effect such publishing will have on Indian science.

Two opposite, extreme views are possible. We shall content ourselves with presenting them here. The first is that while we did have a sprinkling of really outstanding scientists before independence the general level of science and scientific research was increased only later mainly by the intense efforts of a growing and effective scientific community. Now that we are within hailing distance of being amongst the better ones in the world of science we must not do anything that will impede this progress. The publication of foreign science and technology journals in India may undermine not only the efforts to build up our own journals but even the formation of a cohesive and committed scientific community.

The other view is that if the scientists of India are to participate in the global scientific and technological activity they can do so only as equal intellectual partners. Any other stand would reduce us to a position of 'also-rans' so that we will always be followers, and so become scientific sub-contractors of scientists in advanced countries. Hence, if our scientific (and technological) activities have to be on par with international standards, we cannot and should not prevent the publication of international journals in India. The comparison of quality on home ground will egg us on to greater efforts of improving the quality of our science and of our journals.

One can therefore easily visualize the problems that DST will face in advising Government on this ticklish issue. The Department of Science and Technology felt that it may be appropriate to obtain the views of a cross-section of the Indian scientific community through Current Science. We are therefore bringing out the following questionnaire (see following pages) and earnestly urge our readers to return completed questionnaires to Current Science as early as possible.

—*Editor*

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QUESTIONNAIRE—POLICY ON INDIAN EDITIONS OF FOREIGN S&T PERIODICALS

The Government of India's policy on the publication of editions of foreign science and technology periodicals/magazines in India since the mid-fifties can be stated as follows:

> 'So far as the ownership of newspapers and periodicals by nationals of other countries was concerned, the problem was not a very serious one. . . . No foreign-owned newspaper or periodical should, in future, be permitted to be published in India. The recommendation made by the Press Commission, that foreign newspapers and periodicals which dealt with news and current affairs should not be allowed to bring out Indian editions, should be accepted in principle. As far as the "specialized and technical" journals were concerned, the Government did not consider it appropriate to allow such publications.'

Please indicate your views on the following:

I. 1. The present policy, of not allowing S&T magazines to bring out editions in India, is quite sound and ought to continue.

☐ I agree
☐ I do not agree

2. In scientific and technical fields, we will only benefit by allowing publication of editions of good foreign magazines/ journals in India since many more people, who do not presently have access to good libraries or similar other sources, will be able to get the latest information. This will not have any effect on similar indigenous S&T publications; in fact, it may help raise their standards. In any case, our own comparable magazines/periodicals, if they are any good, ought to be able to stand a little competition.

☐ I agree
☐ I do not agree

3. Our own effort in this area, i.e. Indian S&T magazines and journals, has not yet fully matured either in terms of quantity or coverage—largely because the number of S&T personnel even now constitutes only one per cent of our literate population. Allowing foreign magazines/journals to bring out editions in India will be deleterious to our indigenous efforts and may even result in undoing what has taken us many years of sustained efforts to build. If any exception is to be made, that ought to be done on a case-by-case basis after careful consideration of the corresponding indigenous effort, if any.

☐ I agree
☐ I do not agree

II. Some specific suggestions:

1. Indian editions of journals like *New Scientist, Scientific American, La Recherche*, etc., which popularize some of the latest scientific work done outside India but which do not report original contributions.

☐ Yes
☐ Also in Indian languages
☐ No

2. Indian editions of journals like *Nature, Science, Naturwissenschaften*, which not only publish the latest original research and contain original scientific communications, but also discuss scientific discoveries, and present commentaries, news, views, etc.

☐ Yes
☐ No

3. Indian editions of journals from learned societies, academies and institutes containing original papers, like *Physical Review, Physical Review Letters, Journal of Molecular Biology, Journal of Solid State Chemistry* and journals in other subjects.

☐ Yes
☐ No

4. I recommend that Indian editions of the following journals be brought out:

(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
(9)
(10)

5. I would like to add the following remarks/comments: (Please use additional sheets if necessary)

Name ______________________________ Signature:
Position ______________________________ Date:
Address ______________________________
____
____
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(10)

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Name ______________________________ Signature:
Position ____________________________ Date:
Address ____________________________

CURRENT SCIENCE—50 YEARS AGO

ARCHAEOLOGY IN INDIA*

THE report on the work of the Archaeological Survey of India, recently drawn up by Sir Leonard Woolley at the request of the Central Government, should prove extremely helpful to the advancement of knowledge of India's past history....

It points out that "Archaeology could and should play a far more important part than it does in the life of the Indian people. In a country where the historical sense has been but little developed, where historical knowledge is very limited and is then confined for the most part to that political history in which the bias of party feeling tends rather to the perpetuation of old animosities than to the consciousness of national unity, the study of the growth of civilization is the most salutory of all studies. Especially for an Indian at the present time is it important that he should learn how the India which he knows has come into being, understand what is in the light of what has been, and in the clashes of race and creed should stress not the accidents of strife but the power of the Indian spirit to assimilate what was good, even in an enemy culture, without sacrificing its own individuality. Here archaeology can help and the Archaeological Survey as the official organ of archaeology has a duty to perform." This is the point of view from which the whole report has been prepared.

To achieve such ends far more attention needs to be paid to the interpretation of archaeological remains than is the case at present, a coherent plan of campaign for filling the largest and most serious of the many gaps in Indian archaeological history should have precedence over continuance of work on sites already comparatively well known, and publication of results needs to be so reorganized as to bring the Department into closer touch with the general public. These and the provision of adequate training are the fundamental recommendations made, but they all involve considerable modification of present practice, and the modifications needed are carefully considered.

... "Training and tuition are essential. Unwillingly but inevitably I have been forced to the conclusion that this cannot be got in and from the Department as at present constituted. In the matter of the conservation of standing monuments the Department, following carefully the precedents laid down in the past, has done and is doing admirable work to which I can give unstinted praise; only where the preservation of excavated buildings is concerned, for which the precedents are more doubtful, have they indulged in an exaggerated policy which is wasteful financially and its results scientifically deplorable. In the matter of excavation I have on most sites which I have visited found that the methods employed were bad, trained observation conspicuous by its absence, and the results in consequence incomplete and untrustworthy. In the matter of museum direction the report of Messrs. Markham and Hargreaves is sufficient condemnation; the work is amateurish throughout and is seldom informed by any idea of the purposes which a museum should serve. If the present efforts of the Department can be so characterized it is manifest that the staff, before it can train others, must itself be trained. . . ."

On pp. 4–7 the most promising sites for immediate excavation are discussed, and it is pointed out in conclusion that before sites can be selected with really adequate knowledge "there is an enormous amount of preliminary work to be done. Assisted by Circle Officers the archaeological staff should from now on be busied in preparing an archaeological survey of the whole country. Sites of every kind should be visited, listed, mapped, and a carefully made selection of potsherds, coins, etc. collected from each should be filed for identification when the results of excavation make identification possible. I have ventured to recommend sites for excavation on the strength of the study of gazetteers, archaeological reports and such personal investigation as I have been able to make in the course of three months; but the Director-General ought to have at his disposal a detailed survey which would enable him to draw up a programme for future work calculated most surely and most economically to answer to the varying demands of advancing knowledge. Virtually nothing has yet been done in this direction. The task is one requiring organization and will not yield immediate results, for until the collections of pottery etc. can be assigned to their place in the type-sequence which only excavation can establish, the sites must remain undated; but the

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*Extracted from *Curr. Sci.*, 1939, **8**, 397.

material will be there and the filed information will be of permanent value; but it is one which cannot be taken in hand too soon." The establishment of a pottery sequence—or it may be several such sequences for different parts of the country—is considered to be probably the most immediate need, for without it the dating of sites and of the various strata they reveal will in most cases remain impossible.

Another line that needs to be taken up at once is the investigation of Stone Age remains. "India is one of the richest countries in the world for remains of the earliest phases of man's existence.... The efforts of keen amateurs such as Foote only emphasized the importance of the problems to be solved; recently a foreign mission has done some pioneer work in the North-West; but it is not too much to say that this rich field has been entirely overlooked by the Archaeological Survey of India and remains unknown to science. The neglect of it by the Archaeological Survey is due to the fact that the study of Stone Age antiquities is highly specialized and lies quite outside the scope of the ordinary archaeologist; it requires a particular type of training, geological knowledge, etc. and no member of the Survey's staff is at all capable of dealing with it. For this very necessary branch of work to be undertaken specialists must be engaged, and so vast is the field that such should form a permanent element of the staff."

If anything like adequate attention if to be paid to the almost unlimited number of sites that seem likely to repay excavation the Archaeological Survey, even if much more liberally financed than at present, will need all the outside help it can get. It should co-operate with universities and museums, and do all in its power to attract expeditions from outside the country. But in doing so it must insist that nothing is excavated except directly under fully qualified expert supervision, and that the country's interests are adequately safeguarded in the matter of the removal of antiquities. In regard to the latter, evidence is given of the need for tightening up existing practice in certain respects and for a much greater generosity in others, and amendments to the Ancient Monuments Preservation Act are suggested both in regard to these matters and to private ownership of and trading in antiquities (pp. 9-16). The most interesting and important objects should only be preserved on the site when, as at Sarnath near Benares, this is easily accessible from some important centre of population, never when it is inaccessible as at Nagarjunakonda. And it should be the aim of the Department to see that the various types of sculpture that have developed at various times in various parts of the country are well represented in museums, both in India and abroad, provided they will be safely preserved and properly utilized for public education. Only first class specimens can worthily represent India's achievements, and in their absence from foreign museums the country will not get a reasonable chance of receiving the appreciation it deserves.

. . .

The report contains valuable suggestions some of which—such as wider co-operation with other institutions—had already been initiated by the present Director-General before it was written. It is to be hoped that its strong wording will not be allowed to interfere with the careful consideration that it obviously deserves, and that the Central Government will not fail to provide the funds necessary to initiate all such developments as this consideration may show to be desirable. India is so large a country that it is impossible to stop or even adequately check the destruction now going on of scattered megalithic antiquities, sculptures, etc. (most of them still unknown to investigators) by road menders, builders, amateur excavators, etc. And if the other alternative is adopted and all but the conservation side of the Department closed down for a time, much of the priceless evidence now available will inevitably have disappeared before it can be investigated and recorded.

ON THE LIFETIME OF LOW MASS X-RAY BINARIES

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ABSTRACT

It is argued that the measured mass of the binary millisecond pulsar PSR 0021-72A provides a strong evidence that a rapidly spinning neutron star may drastically curtail the X-ray lifetime of a binary system.

In this paper we wish to point out an interesting implication of the measured mass of the recently discovered millisecond pulsar PSR 0021-72A in 47 Tucanae[1,2] as it suggests a possible resolution of one of the outstanding difficulties with the standard spin-up scenario for millisecond pulsars, and in particular, the evolutionary history of this pulsar.

Before stating the problem, we wish to briefly recall some of the properties of this pulsar (table 1). The spin period of the pulsar is ~4.5 ms; the period derivative has not yet been measured. The orbital period is ~32 min and the projected semi-major axis of the orbit of the pulsar in only ~714 km. Of particular interest to the subsequent discussion is the very large eccentricity (~0.32) of the orbit, which suggests that this must be a fairly young system. Given such a tight binary, one expects several general relativistic effects to be very pronounced. Indeed the measured rate of precession of the periastron ($\sim 0°.6\ \text{day}^{-1}$) is 50 times larger than that for the Hulse–Taylor pulsar PSR 1913+16. Ables *et al.*[2] have also measured the Einstein time delay, which is ~510 μs. The measurement of these two general relativistic effects has made possible the determination of two of the most important parameters of the binary system, namely the inclination of the orbit normal to the line of sight and the mass of the pulsar as well as that of its companion. It is remarkable that the orbit is viewed almost exactly face-on ($i = 0.38 + 0.01$ deg). The mass of the companion, presumably a white dwarf, is surprisingly large ($\sim 0.8\ M_\odot$). These two characteristics, and the large orbital eccentricity, have important implications which we shall now discuss.

Table 1 *Parameters of PSR 0021-72A*

Spin period	P	4.4789545(1) ms
Period derivative	$\dot{P}$	$< 10^{-15}\ \text{s s}^{-1}$
Dispersion measure	DM	$67 \pm 2\ \text{cm}^{-3}$ pc
Flux density at 430 MHz	S_{430}	4 ± 2 mJy
Projected semi-major axis	$a_p \sin i$	714 ± 9 km
Binary period	P_b	1942.0845 (20) s
Eccentricity	e	0.32 (1)
Apsidal rate	$\dot{\omega}$	0.565 (20) deg/day
Amplitude of Einstein delay	γ	510 ± 30 μs
Pulsar mass	m_1	$1.38 \pm 0.08\ M_\odot$
Companion mass	m_2	$0.77 \pm 0.05\ M_\odot$
Mean separation	a	300900 ± 4000 km
Orbital inclination	i	0.38 ± 0.01 deg

[From Ables *et al.* 1989]

Numbers in parentheses represent uncertainties in the last digits.

According to the standard picture, millisecond pulsars with low magnetic fields are spun up due to mass transfer in low mass X-ray binaries (LMXBs). The fact that three of the four millisecond pulsars in the galactic disk are in binaries with low-mass companions and circular orbits lends credibility to this hypothesis. The highly eccentric orbit of the pulsar in discussion therefore poses a problem. Also, the fact that one is viewing the orbit almost exactly face-on suggests that the spin axis of the pulsar is inclined at a fairly large angle with respect to the orbit normal; in a close binary in which there has been a mass exchange for a very long time ($\sim 10^8$ years) one should expect the spin axis to be aligned with the orbit normal. If the millisecond pulsar was indeed spun up by accretion then the large mass of its companion also poses a difficulty. Detailed evolutionary calculations[3] suggest that it is difficult to reconcile the measured mass of the companion with the duration of the mass transfer phase necessary to spin it up to a few milliseconds. Because of these difficulties one can rule out the possibility that PSR 0021-72A was spun up in the *presently* observed binary system. Ables *et al.*[2] and Wijers[4] have argued that the most plausible evolutionary route for the present system may be the following. The present system must be the result of a resonant exchange collision between a relatively wide binary with a circular orbit, in which the pulsar was spun up, and a $\sim 0.8\ M_{\odot}$ white dwarf. The very large eccentricity of the orbit and the fact that the spin axis of the neutron star is misaligned with the orbit normal can then be easily reconciled. While the arguments leading up to this conclusion are quite compelling, there is also a serious difficulty. Even if one grants that an exchange encounter can happen once in a Hubble time in a dense cluster such as 47 Tucanae, the 'phase space' for producing a binary with a very large eccentricity ($e \sim 0.99$ even if the encounter took place only $\sim 10^8$ years ago) is likely to be very small[5]. This would then imply that there must be a large number of binary millisecond pulsars as well as several low mass X-ray binaries in this cluster[6]. Although the presence of many binary millisecond pulsars cannot be ruled out at present, the absence of LMXBs poses a problem (though there is one known X-ray source in this cluster, its low luminosity, $\sim 10^{33}$–$10^{34}\ \mathrm{erg\ s^{-1}}$, argues against it being a standard LMXB). This can only be reconciled if the lifetime of LMXBs is much less than is commonly believed. In the standard evolutionary models[3] for LMXBs the mass transfer at near-Eddington rate ($\sim 10^{-8}\ M_{\odot}\ \mathrm{yr^{-1}}$) is expected to be sustained for $\gtrsim 10^8$ yr. Thus in order to drastically reduce the lifetime of the X-ray phase one must prevent the matter from the Roche-lobe overflowing companion from accreting onto the neutron star.

A mechanism for doing this has already been suggested by Ruderman *et al.*[7] After the neutron star accretes for about 10^7 yr at the Eddington rate, it will be spun up to a period ~ a millisecond, and the speed-of-light cylinder will be only a few stellar radii away. Let us now suppose that there are instabilities that cause large modulations in mass transfer rate, such that during a phase of low mass transfer, the Alfvén radius is pushed outside the light cylinder. Once this happens, as Ruderman *et al.*[7] have argued, the pressure of the low-frequency radiation from the millisecond neutron star will permanently quench the accretion of matter onto it. From then onwards the mass lost by the companion will be expelled from the system. Under these circumstances the lifetime of the X-ray phase would be the same as the spin-up time-scale, i.e. $\sim 10^7$ yr.

A similar problem has also arisen in the galactic disk. If LMXBs are the progenitors of millisecond pulsars then the ratio of the number of millisecond pulsars to that of LMXBs should be in the ratio of their lifetimes. While there are at most ~ 100 LMXBs in the disk, the number of millisecond pulsars has been estimated[8] to be $\gtrsim 10^5$. Therefore, even if millisecond pulsars live for ~ a Hubble time, there is an apparent problem if the lifetime of LMXBs is $\gtrsim 10^8$ yr as is generally believed. This has led Kulkarni and Narayan[8] to suggest that there might be other formation mechanisms for millisecond pulsars. However, this difficulty can be overcome[9] if the active lifetime of LMXBs is smaller by a factor of ~ 10 compared to the standard estimates.

If the mechanism suggested by Ruderman *et al.*[7] is the relevant one for reducing the lifetime of the X-ray phase, then there is one important implication for the mass of a spun-up millisecond pulsar. If the accretion phase lasts for $\sim 10^8$ yr, then the mass of the pulsar should be $\sim 2\ M_{\odot}$, whereas if the mass transfer stopped soon after spin-up, it should be ~ 1.4–$1.5\ M_{\odot}$. It is remarkable that the measured mass[2] of the millisecond pulsar PSR 0021-72A in 47 Tucanae is very close to $1.4\ M_{\odot}$. Hence, if this pulsar was indeed spun up in a mass transfer binary, it provides strong evidence for a relatively short lifetime of LMXBs (through the mechanism suggested by Ruderman *et al.*[7]), thereby strengthening the case for LMXBs as the progenitors of binary millisecond pulsars.

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POLAROGRAPHIC AND CYCLIC VOLTAMMETRIC REDUCTION OF *o*-CHLOROBENZALDEHYDE ISONICOTINOYLHYDRAZONE

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ABSTRACT

Polarographic and cyclic voltammetric studies of *o*-chlorobenzaldehyde isonicotinoylhydrazone were carried out in acid and alkaline solutions. The compound shows two polarographic waves in solutions of pH 2–9. In cyclic voltammetric studies, the hydrazone exhibits three cathodic peaks in acid solutions and two cathodic peaks in alkaline solutions. On the basis of these observations it is proposed that the compound undergoes reductive cleavage of =N–N–linkage, followed by the electrochemical reduction of the amide and the imine, the products of the cleavage, to alcohol and amine respectively.

POLAROGRAPHIC and cyclic voltammetric studies of carbonyl derivatives of isonicotinoylhydrazide were undertaken in view of their wide medicinal importance[1,2]. The results obtained for *o*-chlorobenzaldehyde isonicotinoylhydrazone (*o*-Cl-BAINH) are reported here.

EXPERIMENTAL

The compound (*o*-Cl-BAINH) was prepared by the method reported in the literature[3] and purified by repeated recrystallization from hot aqueous alcohol. The hydrazone was confirmed from infrared spectral data and melting point (observed value 222°C, corresponding to the value given in the literature, 221–222°C).

Current–voltage curves were recorded in Britton–Robinson buffer solutions (pH 2–9). A DC pen recording polarograph (model CL-25, ELICO Private Limited, Hyderabad, India) was used to record current–voltage curves. The dropping mercury electrode had the following characteristics: $t = 3.0$ S, $m = 1.93$ mg S^{-1} at a height of 85 cm of mercury column in water under open circuit conditions. Cyclic voltammograms were recorded with a polarographic analyser (model 174 A, Applied Research Company, Princeton, USA) coupled with an X–Y recorder (model RE 0074) and Universal Programmer (model 175). Hanging mercury drop electrode (Metrohm model 9323), platinum wire electrode and SCE were used as working electrode, counter electrode and reference electrode respectively. The results are presented in tables 1 and 2 and figure 1.

RESULTS AND DISCUSSION

Eelectrochemical reduction in acid solutions

The hydrazone (*o*-Cl-BAINH) shows two polarographic waves in acid solutions. The limiting current

Table 1 *Polarographic half-wave potentials of o-chlorobenzaldehyde isonicotinoylhydrazone*
Medium, 20% aqueous DMF
[o-Cl-BAINH] = 4 × 10⁻⁴ M

pH	$-E_{1/2}$ V vs SCE First wave	Second wave
2.0	0.75	0.87
3.0	0.82	0.97
4.0	0.87	1.02
5.0	0.92	1.08
6.0	0.99	1.16
7.0	1.07	1.20
8.0	1.14	1.26
9.0	1.18	1.29

Table 2 *Peak potentials of o-chlorobenzaldehyde isonicotinoylhydrazone*
Medium, 20% aqueous DMF
[o-Cl-BAINH] = 4 × 10^{-4} M

Sweep rate V S^{-1}	$-E_{PC_{I\,V}}$	$-E_{PC_{II\,V}}$	$-E_{PC_{III\,V}}$
pH 4.0			
0.050	0.89	0.99	1.24
0.100	0.91	1.00	1.25
pH 8.0			
0.050	1.17	1.22	—
0.100	1.18	1.30	—

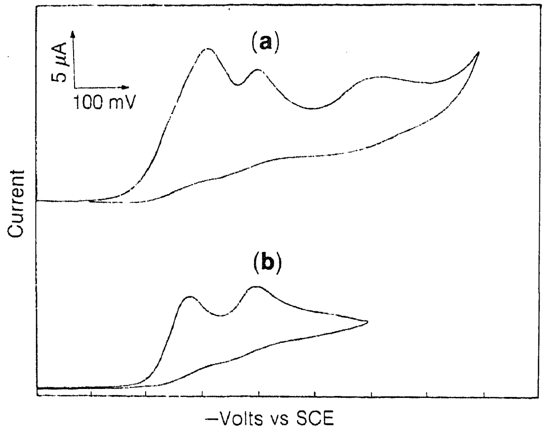
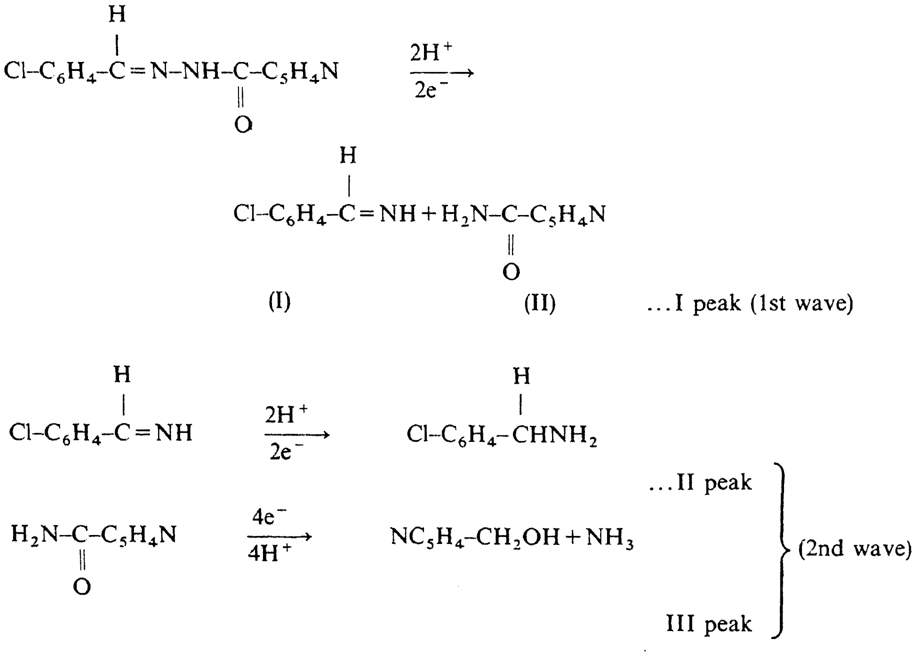


Figure 1. Typical cyclic voltammograms of *o*-chlorobenzaldehyde isonicotinoylhydrazone. **a**, at pH 4.0 (initial voltage, −0.6 V; sweep rate, 100 mV s^{-1}). **b**, at pH 8.0 (initial voltage, −0.9 V; sweep rate, 100 mV s^{-1}).

varied linearly with the concentration of the hydrazone and with the square root of the mercury column height, indicating that the wave is diffusion-controlled. The half-wave potential shifts towards more negative potentials with increase in concentration of the depolarizer, showing that the electrode process is irreversible. This is further confirmed by semilogarithmic analysis. The half-wave potential shifts towards more negative potentials with pH indicating that protons are involved in the reduction process.

The cyclic voltammogram of the compound shows three cathodic peaks in acid solutions in the forward scan; in the reverse scan no peak is observed. This indicates the irreversible nature of the electrode process. This is also confirmed by the negative shift in the peak potentials with increasing sweep rates.

The total number of electrons involved in the polarographic reduction process is established coulometrically to be two and six for first and second waves respectively. The first wave is attributed to the reductive cleavage of =N–N– linkage involving two electrons. The second wave is attributed to the reduction of the imine and the amide to amine and alcohol respectively. The fact that a single wave is observed shows that the two reductions are taking place at almost the same potentials. This is in tune with the reported[4] behaviour of semicarbazones (compounds bearing structural relationship with the present compound). For the purpose of product analysis bulk electrolysis was carried out. However

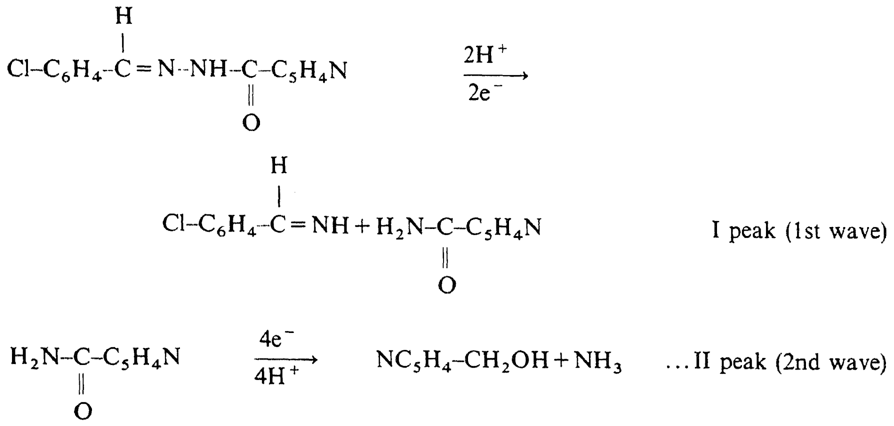


$$\text{Cl–C}_6\text{H}_4\text{–}\overset{\text{H}}{\text{C}}\text{=N–NH–}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{–C}_5\text{H}_4\text{N} \xrightarrow[2e^-]{2H^+} \underset{\text{(I)}}{\text{Cl–C}_6\text{H}_4\text{–}\overset{\text{H}}{\text{C}}\text{=NH}} + \underset{\text{(II)}}{\text{H}_2\text{N–}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{–C}_5\text{H}_4\text{N}}$$

$$\text{Cl–C}_6\text{H}_4\text{–}\overset{\text{H}}{\text{C}}\text{=NH} \xrightarrow[2e^-]{2H^+} \text{Cl–C}_6\text{H}_4\text{–}\overset{\text{H}}{\text{C}}\text{HNH}_2$$

$$\text{H}_2\text{N–}\underset{\text{O}}{\underset{\|}{\text{C}}}\text{–C}_5\text{H}_4\text{N} \xrightarrow[4H^+]{4e^-} \text{NC}_5\text{H}_4\text{–CH}_2\text{OH} + \text{NH}_3$$

Scheme 1



$$\mathrm{Cl{-}C_6H_4{-}\overset{\overset{\displaystyle H}{|}}{C}{=}N{-}NH{-}\underset{\underset{\displaystyle O}{\|}}{C}{-}C_5H_4N} \xrightarrow[2e^-]{2H^+} \mathrm{Cl{-}C_6H_4{-}\overset{\overset{\displaystyle H}{|}}{C}{=}NH + H_2N{-}\underset{\underset{\displaystyle O}{\|}}{C}{-}C_5H_4N} \quad \text{I peak (1st wave)}$$

$$\mathrm{H_2N{-}\underset{\underset{\displaystyle O}{\|}}{C}{-}C_5H_4N} \xrightarrow[4H^+]{4e^-} \mathrm{NC_5H_4{-}CH_2OH + NH_3} \quad \text{...II peak (2nd wave)}$$

Scheme 2

isolation of the products could not be carried out because of low yields. Therefore the catholyte was concentrated and extracted with ether and the extract was tested chemically for the functional groups. The extract gave positive tests for the primary amine (diazotization and coupling with β-naphthol) and for the primary alcohol (oxidation and condensation with 2,4-dinitrophenylhydrazine). The mechanism shown in scheme 1, is therefore proposed for the reduction of *o*-Cl-BAINH.

Electrochemical reduction in alkaline solutions

The hydrazone (*o*-Cl-BAINH) in alkaline solutions shows two polarographic waves. The waves are diffusion-controlled as indicated by the effect of mercury column height and concentration of the hydrazone on the wave height. Semilogarithmic analysis shows the wave to be irreversible. The compound shows two cathodic peaks in the forward scan of the cyclic voltammogram; in reverse scan no peak is observed. This suggests that the electrode process is an irreversible one. This is further confirmed by the negative shift in the peak potentials with increasing sweep rates.

It was reported in the literature that the imine formed during the reductive cleavage of =N–N– linkage in semicarbazones is resistant to further reduction[5]. Further, the reduction of the imine takes place only in the protonated form. The absence of imine reduction wave in alkaline solution is therefore attributed to a significant decrease in the recombination rate. It was reported that amides undergo reduction to the corresponding alcohols[6]. The formation of alcohol as well as the aldehyde in alkaline solutions was confirmed by bulk electrolysis and subsequent indentification. The aldehyde formed is attributed to the decomposition of Ar–CH=NH to Ar–CHO in alkaline solutions. The first wave thus represents the =N–N– reductive cleavage and the second wave the reduction of the amide. The fact that the protonated amide is susceptible to the reduction substantiates the shift in half-wave potential towards negative values in alkaline medium. The observation of two peaks in cyclic voltammograms at potentials equal to the polarographic half-wave potentials supports the mechanism proposed for the polarographic reduction (scheme 2).

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THIOSEMICARBAZONES AS ANALYTICAL REAGENTS: A SELECTIVE SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM

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ABSTRACT

Salicylaldehyde thiosemicarbazone has been proposed as a new reagent for the rapid and selective estimation of 1.0–7.2 ppm of palladium. The reagent instantaneously and quantitatively forms a yellow-orange species with palladium at room temperature in the pH range 1.8–4.0. The coloured species is extractable in oxygenated solvents. Various photometric parameters have been worked out for quantitative estimation of palladium(II) and possible analytical applications (the estimation of palladium in hydrogenation catalysts, dental alloy, jewellery metal, electrical standard resistor and thermocouple) have been reported. The reliability of the method has been worked out with the aid of statistical analysis.

INTRODUCTION

THIOSEMICARBAZONES containing aldehydes or ketones have been employed as analytical reagents for the estimation of metal ions, including platinum group elements[1–6]. However, most of these reagents have limited usefulness for the estimation of palladium, owing to lack of sensitivity, complex formation at elevated temperature, stability of complex with time, more interference from other associated metal ions, etc.

In this paper a method for the spectrophotometric determination of palladium is described. The method has been used in the determination of palladium in small samples of hydrogenation catalysts, dental alloy, jewellery metals, electrical standard resistor and thermocouple.

This photometric method is based on the instantaneous formation of a yellow, water-insoluble Pd(II) complex of salicylaldehyde thiosemicarbazone (SATSC), which is easily extractable into oxygen-containing solvent in the pH range 1.8–4.0.

MATERIALS AND METHODS

Reagents and apparatus

A suitable concentration (~0.01 M) of stock solution of palladium(II) was prepared from $PdCl_2$ (Arora Metthey, India) in minimum volume of 0.1 M HCl and standardized gravimetrically by DMG method[7]. The stock solution was further diluted suitably according to experimental requirements. Standard solutions of diverse ions were prepared from reagent-grade salts; the noble metal compounds were standardized by adopting well-establishe procedures[8, 9]. For the interference study, alkali salt were used for solutions of the anions, and nitrate and chlorides for solutions of the cations. SATSC (m.p. 235°C) was synthesized by the procedure o Sah and Daniels[10]. A stock solution of SATSC (0.01 M) was prepared in ethanol. Walpole buffe solutions were prepared by mixing 0.1 M HCl and 0.1 M sodium acetate to maintain the desired pH values. Absorbance measurements were performed in a Varian model DMS-80 UV/vis spectrophotomete provided with 1 cm matched quartz cells. The pH measurements were made with an Elico digital pH meter model LI 120 provided with a glass–calome combination electrode.

Spectrophotometric determination of palladium

To a solution containing 1.0–7.2 ppm of palladium(II), 3 ml of buffer (pH 3.0 ± 0.1) and 1 ml o 0.01 M reagent were added. The mixture was shaken for 1 minute. To this 10 ml of cyclohexanol wa added and the mixture was shaken vigorously fo 2 min. The two phases were allowed to separate; the organic phase separated was dried over sodium sulphate and the absorbance measured at 405 nm against reagent blank.

Spectrophotometric determination of palladium in hydrogenation catalysts

An accurately weighed sample was dissolved in nitric acid or aqua regia, according to the support and heated on a water bath, if necessary. The cooled

Solution was diluted to 100 ml in a calibrated flask. Suitable aliquots were analysed for palladium as described above.

RESULTS AND DISCUSSION

Absorption spectrum of Pd(II)–SATSC extracted into cyclohexanol

The complex in cyclohexanol was found to absorb maximally at 405 nm; the reagent has absorption maximum at 352 nm. All the present studies were made at 405 nm, where the reagent showed negligible absorption. The absorption maximum does not shift with the changing concentration of palladium(II).

Optimum conditions for Pd(II)–SATSC extraction

The influence of pH over the extraction percentage was studied using an aqueous solution of 4 ppm of Pd(II) with 1 ml of SATSC (0.01 M) and extracting each time with an equal volume of organic phase. The remaining palladium in aqueous phase after extraction was determined by applying the recommended procedure. The results showed that the extraction was almost null for pH lower than 1.0, whereas the extraction was complete in the pH range 1.8–4.0. Of the various oxygen-containing solvents for extraction tried, cyclohexanol was found to be more effective since the complex showed maximum absorbance in this solvent. Shaking for 30 sec was sufficient. An at least 2-fold excess of reagent was necessary for maximum colour development, and even a 100-fold excess had no adverse effect. The order of addition of reagent did not affect the absorbance of the complex. There was no influence on the absorbance when complexation was effected at higher temperature. Cyclohexanol extract remained spectrophotometrically stable for 24 h. The influence of aqueous : organic phase volume ratio on the absorbance was studied to increase the sensitivity of the method proposed. It was observed that a v_w/v_0 ratio of 5 allows the quantitative determination of lower concentrations of palladium in the aqueous phase. Since the reagent (SATSC) was prepared in ethanol, the permissible ethanolic content in aqueous phase should be less than 35%; beyond 45% the two phases were miscible in all proportions.

Stoichiometry of the extracted complex

A simple graphical test was adopted for confirming single or multiple species formation. As per Coleman's analysis[11]; a series of sets of wavelengths were selected from the absorption spectra and the absorbance values obtained for each set of wavelengths at varying Pd(II) concentration were plotted. Linear plots passing through the origin confirm single species formation. The combining ratio of Pd(II) with SATSC was investigated by Job's method[12] and further verified by mole-ratio method[13] as 1:1. The isolated solid complex was subjected to various physicochemical studies[3], which indicated that the complex is $[Pd(II)(SATSC)Cl_2]$.

Beer's law range and sensitivity of the method

The system obeys Beer's law at 405 nm over the range 1.0–7.2 ppm of palladium. The molar absorptivity of the system was found to be 6.4×10^3 mol^{-1} cm^{-1}. The corresponding Sandell's sensitivity of the method was 0.017 $\mu g/cm^2$. The optimum concentration range, evaluated by Ringbom's method, was 1.3–6.5 ppm. The formation constant of Pd(II)–SATSC was calculated to be 3.3×10^8 from the curve obtained from Job's method.

Interference by other ions

The recommended procedure was followed to study interference due to various ions in aqueous solutions containing 4 ppm of palladium(II). Of the various cations and anions tested individually in the estimation of Pd(II), no interference was observed in the presence of 100, 50, 25, 10, 5 and 2-fold excess of EDTA, NH_4^+, Co(II), Ca(II), Ba(II), chloride, fluoride and nitrate; sulphate, oxalate, Al(III) and acetate; Zn(II), Ni(II) and Mn(II); Fe(II, III), W(VI), Mo(IV), thiocyanate and iodide; Cu(II), Ir(III), Rh(III), Cr(III), Au(III) and Ru(III); and Pt(II) respectively. Thus, by the recommended method, Pd(II) can be determined in the presence of greater number of diverse ions, including moderate amounts of most of those that are commonly associated with palladium in real and synthetic samples (except Pt(II), which can also be overcome by precipitating palladium[14] prior to photometric estimation of Pd(II) as the SATSC complex).

Analytical applications of the method

In order to assess the analytical usefulness of the proposed method, synthetic mixtures simulating the compositions of dental alloy, jewellery metal, electrical standard resistor and thermocouple were made and the palladium content was determined in each (table 1). The method developed was also

Table 1 *Estimation of palladium in dental alloy, jewellery metal, electrical standard resistor and thermocouple*

Metal constituent	Concentration (ppm) I	Concentration (ppm) II	Palladium found* after isolation of Pd(II)	Error (ppm)
Dental alloy				
Palladium	2.0	4.0	2.07 (4.18)	+0.07 (+0.18)
Platinum	2.0	4.0		
Copper	5.0	10.0		
Zinc	5.0	10.0		
Jewellery metal**				
Palladium	2.8	5.6	2.72 (5.67)	−0.08 (+0.07)
Ruthenium	0.2	0.4		
Electrical standard resistor				
Palladium	4.0	6.0	4.20 (5.82)	+0.20 (−0.18)
Platinum	4.0	6.0		
Thermocouple				
Palladium	3.0	6.5	3.25 (6.33)	+0.25 (−0.17)
Platinum	9.0	19.5		

*Average of four determinations. **Direct determination (without isolation of palladium).

applied to the determination of palladium in small samples of hydrogenation catalysts. The results are shown in table 2, and compared with those obtained by atomic absorption spectroscopy (A.A.S).

Reliability of the proposed method

The precision of the present method was evaluated by determining the same amount (40 μg/10 ml or 4 ppm) of Pt(II) in solution in 10 different samples. The mean absorbance was found to be 0.530 with a standard deviation of 0.016. The correlation coefficient was 0.9984, which indicates excellent linear relationship between the measured signal and the true concentration.

Table 2 *Spectrophotometric estimation of palladium with SATSC in hydrogenation catalysts*

Catalyst (Fluka)	Found (%) AAS method	Found (%) Proposed photometric method*
Pd/asbestos	4.40	4.37
Pd/activated charcoal	9.42	9.37
$Pd/CaCO_3$	5.60	5.78
$Pd/BaSO_4$	9.78	9.94

*Average of 5 determinations.

Use of SATSC for the estimation of palladium(I has the following advantages: simplicity, good sensitivity, high stability, insensitivity to temperatur rapidity and very good selectivity. The entir procedure extraction and assay takes only 10–15 min

ACKNOWLEDGEMENTS

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22 September 1988

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ANNOUNCEMENTS

Traction Batteries for Electric Vehicles

Place: New Delhi
Date: 20 and 21 October 1989
Contact: Conference Secretariat
The Battery Society of India
c/o Indian Lead and Zinc Information Centre
No. 7, Shopping Centre, Block B-6
Safdarjung Enclave
New Delhi 110 029

Seminar on University–Industry Interactions

Place: Bangalore
Date: 13 October 1989
Contact: The Convener
Centre for Science and Technology
111/1 (56 new), 6th Main Road
Malleswaram
Bangalore 560 003

The seminar will focus in (i) undergraduate education in engineering and the problems of industries in respect of adequacy of training of students to fit into industry, and (ii) scope for response of industry in terms of training facilities for students etc. Views on these aspects are invited.

International Conference on Artificial Intelligence in Industry and Government

Place: Hyderabad, India
Date: 23–25 November 1989
Contact: Conference Secretariat
National Centre for Expert Systems
Institute of Public Enterprise
Osmania University Campus
Hyderabad 500 007
India

International Conference on Superconductivity

Place: Bangalore, India
Date: 10–14 January 1990
Contact: Prof. S. V. Subramanyam
Chairman, Local Organizing Committee, ICSC
Department of Physics
Indian Institute of Science
Bangalore 560 012
India

SHORT COMMUNICATIONS

MONSOONAL MICROTREMORS AT KOTTULI, CALICUT

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FREQUENT microtremors accompanied by rumbling noises have become part of the monsoonal phenomena since 1984 at Kottuli, Calicut (11°17′25″ N, 75°55′25″ E). Though this is a new experience for the present population, historical reports indicate that such features were not uncommon. Even the name of this village means, 'the sound of the strike'. Almost all of these vibrations took place in July or August (1858, 1959 and 1964). Microseismic observations indicate the shallow origin of these tremors.

The absence of reactivatable faults and reservoirs in the proximity increases the complexities in determining the causes of the tremors. In the case of the tremors of 1 July 1984, which started at 3.47 p.m. along with landslides in the Western Ghats, the relation between the microtremors and the landslides was fixed as genetically simultaneous. The tremor recorded at Kottuli was responsible for the landslide at Meppadi. Photogeological studies indicate the presence of major lineaments tending NW–SE and ENE–WSW, confluencing at Kottuli. This confluence isolates a northern block, which moves over the remaining block to release the confined pressure. The movement is made possible by the water trapped in the lineamental zone. This water system, and its continuation throughout, help transmission of the confined pressure and its release. All the earlier tremors in the Malabar area occurred in July or August, and since confined water is the prime reason for the tremors, the microtremors stop when the monsoon rains are over.

18 November 1986; Revised 21 February 1989

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monsoon

SULPHIDE MINERALIZATION AROUND AMBADUNGAR, BARODA DISTRICT, GUJARAT

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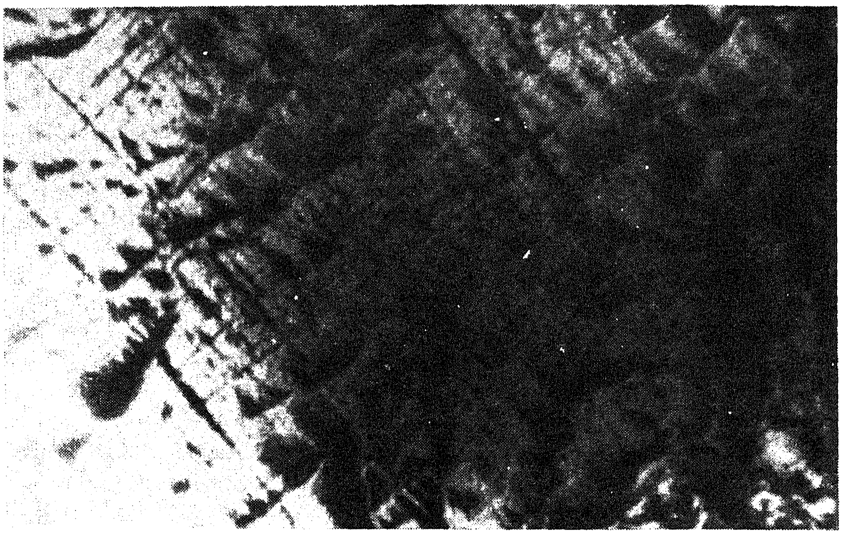
SULPHIDE mineralization around Ambadungar (Survey of India Toposheet No. 46 K/l; 22°1′ N: 74°4′ E) is being reported for the first time. The Ambadungar alkali–carbonatite complex is well known for its fluorite mineralization[1,2]. The proved reserves of fluorite are now close to being exhausted. The purpose of this note is to present the results of the study of the ore-minerals. The stratigraphic succession in Ambadungar complex may be summarized as:

Alluvium	Recent to sub-Recent
Alkaline–carbonatite complex	Eocene and later (37.5 ± 2.5 Myr)[3]
——— Non-conformity ———	
Deccan traps	Late Cretaceous to Eocene/Oligocene (65 to 50 Myr)[4, 5]
——— Minor unconformity ———	
Bagh beds	Cretaceous
——— Major unconformity ———	
Granite gneiss forming basement	Dharwarian[6]

The sulphide mineralization is associated with carbonatites at a deeper level in a tunnel opening about 40 m below the present working bench.

Polished ore sections have been studied following standard texts on determinative aspects[7, 8]. Identified ore species include galena, pyrite, sphalerite and chalcopyrite, while the gangue minerals are fluorite, calcite, quartz and dolomite. Galena shows intergrowth texture with the fluorite. Triangular cracks are filled with sphalerite and pyrite. The large grain size and associated fluorite suggest undisturbed crystal growth in large cavities (figure 1). Cleavage is quite distinct in pyrite and harder than the chalcopyrite and sphalerite (figure 2).



1

2

Figures 1 and 2. Photomicrographs (× 75) of sections of ores from Ambadungar. **1,** Polished galena showing intergrowth texture. Sphaleritic vein is also observed. **2,** Polished pyrite showing cleavage traces. Associated ores are chalcopyrite and sphalerite.

Both Pb and Zn occur as galena and sphalerite in veins and late-stage replacement carbonatitic phases; galena is somewhat more widespread (cf. Kaiserstuhl occurrence)[9]. From trace-element studies[10,11] the high concentrations of Ba, Sr, REE, etc., and of CO_2, F, P, Cl, S, etc. are considered typomorphic for magmatic processes. In local secondary ultrabasic and basic magma chambers, rich in Ca and Mg, large amounts of $CaCO_3$ and $MgCO_3$ are formed in the final phase of magmatic crystallization. Calcite and, rarely, dolomite carbonates terminate intrusive alkali series and the association of alkali basalt magma (picrite) and components of alkaline rocks including nepheline syenite (phonolite) and phono-nephelinite together with components of carbonates are formed in the late stage[12].

The occurrence of sulphides of Pb, Cu, Zn, etc. in substantial amounts indicates a high-temperature (telethermal) environment, and the prospect of encountering more fluorite at deeper levels is negated.

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SPECTROPHOTOMETRIC DETERMINATION OF MICRO QUANTITIES OF GOLD WITH CETYLTRIMETHYLAMMONIUM BROMIDE IN PRESENCE OF IODIDE

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THE reagents [Fe(phenanthroline)$_3^{2+}$][1], tetraphenylarsonium chloride[2], diethyl ether[3], Rhodamine B[4], mesityl oxide[5,6] or primaquine phosphate[7] have been used in spectrophotometric methods for determination of gold. In the present investigation we noted that gold(III) forms an yellow ion-association complex with cetyltrimethylammonium bromide (CTAB) in presence of potassium iodide and is extractable into chloroform. Based on this a simple spectrophotometric method for the determination of gold is reported.

A stock solution of Au(III) was prepared by dissolving gold chloride (Johnson and Matthey) in distilled water and standardizing[8]. Solution of lower concentration of the metal was prepared by appropriate dilution of the stock. Aqueous solutions of CTAB (0.025 M SISCO) and potassium iodide (0.03 m BDH) were used. KH_2PO_4–NaOH buffer was employed to adjust the pH of the aqueous solution. Standard solutions of diverse ions were prepared from their salts. Chloroform and other solvents were distilled before use. All other reagents used were of analytical grade.

Absorbance measurements were made with a Shimadzu PR1 model recording spectrophotometer. Stoppered quartz cells of 10 mm optical path length were used for all measurements. An ECL 5651 digital pH meter was used to measure the acidity of the aqueous solution.

To an aliquot containing Au(III) (70 μg) were added potassium iodide (1 ml) and CTAB (0.1 ml). The mixture was adjusted to pH 7.0 with buffer. The volume of the aqueous phase was made up to 10 ml. The mixture was then equilibrated (30 sec) with chloroform (10 ml). The separated organic layer was dried over anhydrous sodium sulphate. Finally the absorbance of the chloroform extract was measured at 290 or 360 nm against a blank prepared in a similar manner. The amount of gold was computed from a calibration curve. To test for interference by other ions, the ions were added to the system before addition of the reagents.

When potassium iodide was added to a neutral or slightly acidic solution containing gold (III), a yellow coloration due to the formation of $[AuI_4]^-$ was formed. This complex anion was not extractable into chloroform. On addition of an aqueous solution of CTAB to the coloured solution, an ion-association complex, probably of the type $[CTA]^+[AuI_4]^-$, was formed. This was extractable into chloroform. Other solvents like ethyl acetate, benzene and 1,2-dichloroethane were also tested as extracting solvents, but these offered no special advantages over chloroform (table 1). The complex was not extractable into carbon tetrachloride.

The reagent blank itself shows absorption maximum at 250 nm and the absorption becomes

Table 1 *Absorption maxima and molar absorptivities of Au(III)–I–CTAB complex extracted into different solvents*

Solvent	Absorption maxima (nm)	Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$) ($\times 10^{-4}$)	Sensitivity ($\mu g/cm^2$)
Chloroform	290	2.14	0.009
	360	1.29	0.015
Benzene	298	1.97	0.010
	365	1.32	0.014
Ethyl acetate	290	2.08	0.009
	360	1.13	0.017
1,2-Dichloroethane	295	1.77	0.011
	365	1.07	0.018
Carbon tetrachloride*	—	—	—

*Complex not extractable.

insignificant beyond 290 nm. Hence all absorption measurements were made at 290 or 360 nm. The Au(III) complex showed constant and maximum absorbance when the extractions were carried out in the pH range 0–9, yielding quantitative recovery of gold in a single extraction.

The absorbance of the Au(III)–I–CTAB complex in chloroform showed a linear response up to 15 ppm of gold at 290 or 360 nm. The molar absorptivity of the complex on the basis of gold content worked out to be 2.14×10^4 and $1.29 \times 10^4\ l\ mol^{-1}\ cm^{-1}$, corresponding to Sandell's sensitivities 0.009 and 0.015 $\mu g/cm^2$, at 290 and 360 nm respectively. This classifies the colour reaction (stable for 24 h) as one of the most sensitive for gold. Further, with variation of reagent concentration, it was noted that 1 ml of 0.03 M of KI with 0.1 ml of 0.025 M of CTAB was sufficient to extract 70 μg of gold(III) in a single extraction. Increasing the concentration of the reagent did not bring about any significant change in the maximum value of absorbance.

The average of six determinations of 70 μg of Au(III) was 69.5 μg, with a relative mean deviation of 2.87%. The process is very simple and rapid, requiring only 10–15 min for each run.

In interference tests, an ion was considered to interfere if the recovery of gold differed by more than ±3% from the actual amount. In the present method, gold(III) (70 μg) could be determined without interference in presence of 100-fold excess of Cr(III), V(V), Mo(VI), Hg(II), Rh(III), Al(III), Cu(II), Ni(II), Mg(II), Pt(IV), Pd(II), Zn(II), Cd(II), Pb(II), Zr(IV), Ca(II), Ba(II), Sr(II), Th(IV) and La(III). Fifty-fold excess of Fe(III), Co(II), Mn(II), Ag(I) and Bi(III) could be tolerated. U(VI) interfered. Among the anions tested, 200-fold excess of borate, phosphate, tartrate, citrate, fluoride, bromide, phthalate, oxalate, acetate and nitrite, and lower concentrations of ascorbate and EDTA did not interfere in the estimation of gold. Thiocyanate, thiosulphate, thiourea and arsenate interfered.

In the absence of real samples, the applicability of the method was tested by analysing some synthetic mixtures containing Au(III), Ag(I), Cu(II), Zn(II), Fe(III), Co(II), Ni(II), Pd(II), Pt(IV) and Rh(III). The compositions of the mixtures and percentage recovery of gold are given in table 2. The results show that recovery of gold was highly satisfactory. Average of three determinations was taken in each case.

Table 2 *Analysis of synthetic mixtures*

Composition with amounts taken in μg	Recovery of gold (%)
Au(III) 70, Ag(I) 100, Cu(II) 100, Zn(II) 100	98
Au(III) 70, Fe(III) 100, Co(II) 100, Ni(II) 100	98
Au(III) 70, Pd(II) 100, Pt(IV) 100, Rh(III) 100	101

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PHOTO-SENSITIZED OXIDATION OF 6,7,8,9,10,10-HEXACHLORO-1,5,5a,6,9,9a-HEXAHYDRO-6,9-METHANO-2,4,3-BENZODIOXATHIEPIN-3-OXIDE (ENDOSULFAN) BY SINGLET OXYGEN

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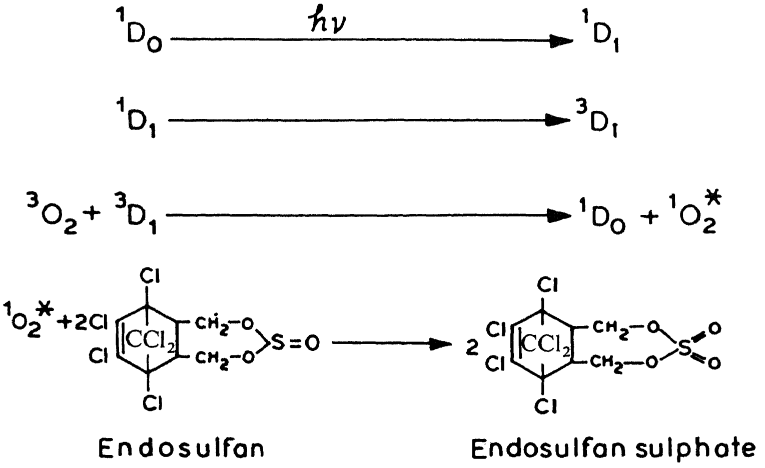
GUNTHER[1] observed that DDT lost its toxic effects quickly when sprayed in the field during summer. It was further studied by various workers[2–5]. Photodecomposition of aldrin and dieldrin was reported by Henderson and Crosby[6], whereas Benson *et al.*[7] characterized the photoproducts of chlordane. Photodecomposition of the chlorinated hydrocarbon endosulfan was observed by Archer *et al.*[8,9], while Dureja and Mukerjee[10] did not observe any photosensitization product.

Endosulfan (technical grade) (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), 1,4-diazobicyclo-(2,2,2)-octane (DABCO), methylene blue (CI–52015) and methanol (BDH) were used. All other chemicals used were of SM grade. Endosulfan was used after recrystallization (m.p. 95°C). A 200 W tungsten lamp was used for irradiating the reaction mixture; IR radiations were cut off using a water filter. The reaction mixture was prepared by adding four drops of methylene blue (2×10^{-6} M) in methanolic solution of endosulfan (8% v/v) and was aerated for 5 h in the presence of light. The progress of the reaction was followed by TLC. Some suspension was observed in the reaction mixture after 3 h.

A white substance that settled in the reaction mixture after 5 h was separated by decantation. The methylene blue was removed by the addition of activated charcoal. Dureja and Mukerjee[10] did not observe any photoproduct in methanolic solution with methylene blue in the presence of light from a tungsten lamp while we have obtained a photoproduct, which has been characterized as the sulphate derivative (recrystallized, m.p. 178°C). The following were the results of elemental analysis: found



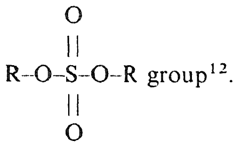
$^1D_0 \xrightarrow{h\nu} {}^1D_1$
$^1D_1 \longrightarrow {}^3D_1$
$^3O_2 + {}^3D_1 \longrightarrow {}^1D_0 + {}^1O_2^*$
$^1O_2^* + 2$
Cl
CCl₂
CH₂–O
S=O
Endosulfan
2
Endosulfan sulphate

Scheme 1

---

*For correspondence

$C = 25.53\%$, $H = 1.50\%$, $Cl = 50.15\%$; and calculated for $C_9H_6Cl_6O_4S$, $C = 25.53\%$, $H = 1.41\%$, $Cl = 50.35\%$. Further, the endosulfan and the photoproduct were hydrolysed with alkali and the hydrolysed solutions gave positive test for sulphite and sulphate ions respectively[11]. The bromo derivatives of endosulfan and its photoproduct were prepared; the m.p.'s were 85 and 55°C, respectively. New bands were seen in the IR spectra of the photoproduct at 1445, 1230 and 645 cm^{-1}, which are characteristic of



$$\mathrm{R{-}O{-}\overset{O}{\overset{\|}{\underset{\underset{O}{\|}}{S}}}{-}O{-}R}\ \text{group}^{12}.$$

However, the NMR spectra of endosulfan and its photoproduct are almost identical. The product of photo-sensitized oxidation of endosulfan was the same as that of oxidation carried out in the dark using $H_2O_2 + NaOCl$ mixture as the oxidant.

It was confirmed that the C=C bond remained unattacked during oxidation[13] and therefore the site of attack in endosulfan is the sulphur atom. The participation of singlet oxygen as the active oxidizing species in the photo-oxidation was confirmed by using singlet oxygen scavengers, where the yield was considerably reduced[14].

The experimental data suggest the mechanism shown in scheme 1. The primary process of the photo-chemical reaction involves the absorption of radiation while the secondary reactions lead to the transformation of the various electronically excited states to give chemical products.

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SYNTHESIS OF SOME 2-AMINO-3-CYANO-4, 6-DISUBSTITUTED PYRIDINE DERIVATIVES AND THEIR ANTIBACTERIAL ACTIVITY

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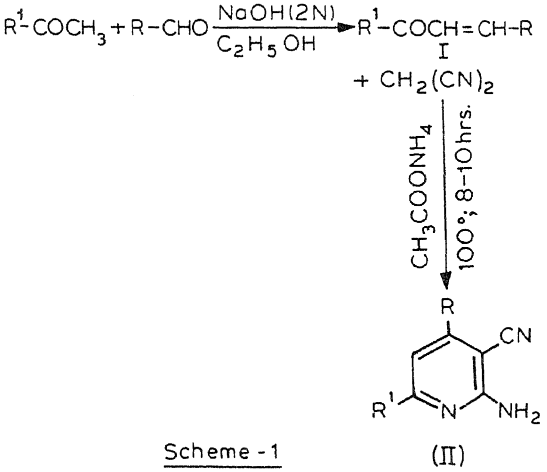
CYCLIZATION of chalcones with dicyanomethane in 1:1 molar ratio in the presence of ammonium acetate gives 2-amino-3-cyano-4, 6-disubstituted pyridines through Michael reaction.

Cyanopyridines with different alkyl and aryl groups have been found to have antimicrobial[1,2], fungicidal[3-5], antihypertensive[6-8], cardiovascular[9], and other activities. Only a few cyanopyridines have been synthesized by Michael reaction[10], and by other methods[11-15], during the past few years. Keeping in view the biological activity of the cyanopyridine derivatives, we have synthesized some new cyanopyridine derivatives.

The condensation of acetophenone (1 mol) with benzaldehyde (1 mol) in the presence of sodium hydroxide and ethanol resulted in the formation of chalcone (I). Dicyanomethane and chalcone reacted in 1:1 molar ratio in the presence of ammonium acetate to give 2-amino-3-cyano-4,6-disubstituted pyridine (II) through Michael reaction with the elimination of 1 mol each of water and hydrogen (scheme 1). All the newly synthesized compounds (II) are yellow and are soluble in chloroform, DMSO and TFA.

IR spectra (KBr pellets) were recorded on a Perkin–Elmer 577 grating infrared spectrophotometer. All the synthesized 2-amino-3-cyano-4,6-disubstituted pyridines showed three characteristic bands in the region 3470–3020 cm^{-1}, which are due to $-NH_2$ group, and a strong band due to $-C\equiv N$ group in the region 2220–2200 cm^{-1} (table 1).



Scheme - 1

^1H NMR spectra were recorded on an FX 90Q JEOL spectrometer (90 mHz) in $CDCl_3$ and in DMSO-d_6 using TMS as an internal standard. The chemical shifts are given in ppm downfield from TMS (table 1).

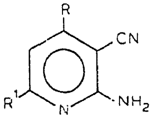
The chalcones were synthesized according to Vogel's method[16].

Synthesis of 2-amino-3-cyano-4,6-disubstituted pyridines

A mixture of dicyanomethane (1 mol), chalcone (1 mol) and ammonium acetate (8 mol) in ethanol (150 ml) was refluxed on a water bath for about 8–10 h. The contents of the flask were poured into crushed ice with constant stirring to obtain a solid yellow mass, which was washed with water and ethanol. the residue was recrystallized from ethanol–dimethylformamide (2:1). The prepared compounds are listed in table 2.

Antibacterial activity: The antibacterial activity of all the compounds was determined according to the method of Varma and Nobles[17]. The test organisms were *Escherichia coli* and *Staphylococcus aureus*. Sterile filter paper discs (5 mm diam.) saturated with the solution of the test compounds were placed on inoculated plates 0.5% NaCl, 0.5% glucose, 2.5% peptone, pH 6.8–7.0, 1.5% agar after allowing solvent to evaporate. The plates were incubated at 37°C for 24 h. Zones of inhibition around the discs were measured. All the compounds exhibited significant activity against *S. aureus* but showed no activity against *E. coli* (table 3).

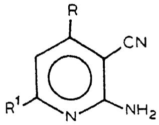
Table 1 *IR and ^1H NMR spectroscopic data for 2-amino-3-cyano-4,6-disubstituted pyridines*



	IR (KBr: ν, cm^{-1})		NMR ($CDCl_3$: δ ppm from TMS)				
Compound	$-NH_2$	$-C\equiv N$ (conj.)	$-OCH_3$	$\equiv CH$ (aromatic)	$-NH_2$	Aromatic protons (R^1)	Aromatic protons (R)
1	3430, 3370, 3040	2210 *s*	—	7.14 *s*	7.30–7.6 *bs*	7.77–8.26	6.5–7.0
2	3430, 3270, 3150	2200 *s*	3.82 *s*	7.37 *s*	7.51–7.78 *bs*	7.79–8.25	6.86–7.26
3	3470, 3330, 3200	2220 *s*	—	7.35 *s*	7.38–7.69 *bs*	7.74–8.19	6.80–7.30
4	3420, 3320, 3020	2200 *s*	—	7.89 *s*	7.91–8.20 *bs*	8.27–8.60	6.90–7.80
5	3460, 3350, 3080	2210 *s*	—	7.35 *s*	7.40–7.74 *bs*	7.76–8.21	6.84–7.11
6	3470, 3330, 3150	2220 *s*	—	7.24 *s*	7.37–7.76 *bs*	7.77–8.11	6.7–7.10
7	3465, 3365, 3155	2220 *s*	3.82 *s*	7.14 *s*	8.30–8.57 *bs*	8.58–8.70	6.97–7.10
8	3450, 3350, 3145	2220 *s*	3.82 *s*	7.03 *s*	7.24–7.70 *bs*	7.71–8.10	6.36–6.9
9	3470, 3365, 3140	2220 *s*	3.82 *s*	7.50 *s*	8.04–8.51 *bs*	8.53–8.70	6.56–7.16
10	3400, 3310, 3150	2180 *s*	—	7.56 *s*	7.78–8.21 *bs*	8.25–8.48	6.75–7.33

s, Strong; *s*, Singlet; *bs*, broad singlet.

Table 2 *Physical and chemical data for 2-amino-3-cyano-4,6-disubstituted pyridines*



Compound	R	R^1	Mol. formula	mp* (°C)	Yield (%)	Analysis, % found (calcd) C	H	N
1	$m.ClC_6H_4$	C_6H_5	$C_{18}H_{12}ClN_3$	226	30	70.90 (70.70)	4.05 (3.93)	14.00 (13.75)
2	$p.OCH_3C_6H_4$	C_6H_5	$C_{19}H_{15}N_3O$	165	36	75.89 (75.75)	5.13 (4.98)	13.92 (13.95)
3	$o.ClC_6H_4$	C_6H_5	$C_{18}H_{12}ClN_3$	190	30	71.10 (70.70)	4.13 (3.93)	13.50 (13.75)
4	$m.BrC_6H_4$	C_6H_5	$C_{18}H_{12}BrN_3$	248	32	62.03 (61.71)	3.66 (3.43)	12.40 (12.00)
5	$p.BrC_6H_4$	C_6H_5	$C_{18}H_{12}BrN_3$	175	34	61.86 (61.71)	3.30 (3.43)	11.66 (12.00)
6	$p.FC_6H_4$	C_6H_5	$C_{18}H_{12}FN_3$	140	50	74.61 (74.74)	4.36 (4.15)	14.93 (14.53)
7	$p.OCH_3C_6H_4$	$p.ClC_6H_4$	$C_{19}H_{14}ClN_3O$	170	35	68.10 (67.96)	4.31 (4.17)	12.27 (12.52)
8	$p.OCH_3C_6H_4$	$p.BrC_6H_4$	$C_{19}H_{14}BrN_3O$	140	34	60.40 (60.00)	3.56 (3.68)	11.37 (11.05)
9	$p.OCH_3C_6H_4$	$p.NO_2C_6H_4$	$C_{19}H_{14}\;{}_4O_3$	220	30	66.21 (65.89)	4.20 (4.05)	16.38 (16.18)
10	C_6H_5	$p.NH_2C_6H_4$	$C_{18}H_{14}N_4$	260	34	75.38 (75.52)	4.80 (4.90)	19.90 (19.58)

*Melting points are uncorrected.

Table 3 *Antibacterial activity*

Compound	*S. aureus*	*E. coli*
1	+ + +	−
2	+ +	−
3	+	−
4	+ +	−
5	+	−
6	+ + +	−
7	+ + +	−
8	+ +	−
9	+ + +	−
10	+ +	−
Gentamycin	+ + + +	+ + + +

−, No inhibition; +, inhibition zone 6–7 mm; + +, 7–8 mm; + + +, 9–11 mm; + + + +, 20–22 mm.

The bacterial cultures were obtained from the Microbiology Laboratories, S.M.S. Medical College, Jaipur.

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5 September 1988

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A NEW SPECIES OF *CHRYSOSPORIUM*

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THE genus *Chrysosporium* Corda has been reviewed earlier[1–3]. Presently 22 species of this genus have been enumerated[3]. *C. tropicum*, *C. lucknowense*, *C. indicum*, *C. evolceanui*, *C. crassitunicatum*, *C. carmichaeli*, *C. queenslandicum* and *C. sulfureum*[4–8] were isolated from Indian soil. The present communication deals with a new species of *Chrysosporium* not described earlier.

Chrysosporium geophilum Kushwaha and Shrivastava sp. nov. (figure 1).

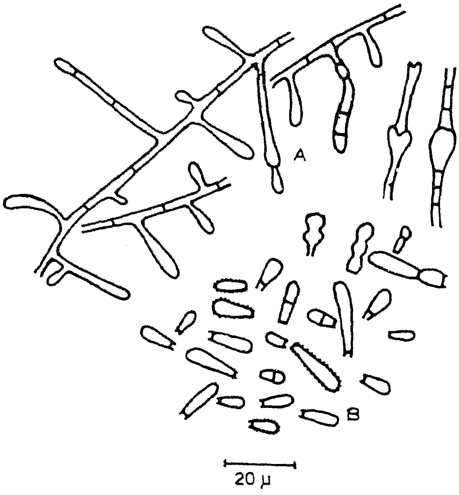

Figure 1. *Chrysosporium geophilum.* **A,** Hyphae, with conidiogenous cells; **B,** conidia.

Colonies in agaro Sabouraudii cum dextroso composite per viginti dies crescentes diametrum 60–70 mm attingentes, albee, subtus pallide cremeobrunneae, densae definito, aequali. Hyphae sepatatae, hyalinae, tenuiter tunicatae, 1–4 µm crassae, frequenter celluas condioigenas inflates, hyellnas, leviter et cresse tunicatas, subglobosas wel aliquantum anormes, 8 µm crassas producentes. Hyphae spatuliformes productac. Conidia terminalia lateraliaque sessilia vel in prominentiis brevibus gests, subhyaline, levia vel aspara, tenuiter tunicatae, abovoidea vel clavata, continus vel raro bicellularia, 2–4 × 4–20 µm, cicatrice basali lato notata 4–10 × 2–4 µm intercalaria rariora, subhyalina, levia vel aspera, doliiformia vel allipsoidea, 2–4 µm.

Colonies on Sabouraud's dextrose agar white, 60–70 mm in diameter, reverse pale creamy brown, dense and fluffy at centre, thinner outwards, raised centrally, margin defined. Hyphae septate, hyaline, thin-walled, 1–4 µm wide, frequently producing swollen conidiogenous cells, hyaline, smooth and thick-walled, subglobose or irregular in shape, up to 8 µm wide. Racquet hyphae present. Lateral conidia sessile on short protrusions, subhyaline, initially echinulate and some becoming smooth-walled on maturity, thick-walled, obovoid to clavate, 1-celled, 4–20 × 2–4 µm. Two-celled conidia rare, 4–10 × 2–4 µm. Intercalary conidia less abundant, subhyaline, smooth or rough, thick-walled, barrel-shaped to ellipsoid 2–4 µm.

This fungus was isolated from human hair buried in soil collected from the L.L.R. Hospital Campus, Kanpur, India, in May 1982 and its specific epithet refers to its habitat.

Subcultures were deposited in the culture collection of the Department of Botany, Christ Church College, Kanpur (CC/534); CMI, Kew, England (IMI 276183); and ITCC, New Delhi, India (ITCC 3345).

This fungus bears some similarities to *C. indicum*, *C. tropicum* and *C. pannicola* in its morphology. However, the present fungus can be differentiated from *C. indicum* and *C. tropicum* on the basis of presence of 2-celled and larger conidia borne on swollen conidiogenous cells. The large conidia of *C. geophilum* which are initially echinulate and become smooth-walled on maturity further differ from those of *C. pannicola*.

Two-celled conidia of *C. carmichaeli* and *Chrysosporium* anamorph of *Arthoderma curreyi* also resemble those of the new species, but on the basis of colony colour, larger conidia and swollen conidiogenous cells *C. geophilum* can be separated from these two fungi. The swollen conidiogenous cells of this species may be regarded as rudimentary ampulliform swellings as in *Chrysosporium pannicola* and *Myceliophthora fergusii*. The present fungus is classified in *Chrysosporium* because of its conidia with broad bases. This fungus grew well on moistened human hair and Sabouraud's dextrose agar at 35°C. The keratinophilic nature of this fungus was further determined by the method of Agrawal and Kushwaha[9].

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LARGE CARDAMOM—A NEW HOST FOR *PESTALOTIOPSIS VERSICOLOR* (SPEG.) STEYAERT

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LARGE cardamom (*Amomum subulatum* Roxb.) is an important cash crop of Sikkim occupying the largest area and highest production in India. During a survey of large cardamom diseases several foliar diseases were noticed. Recently symptoms of leaf blight, which could severely damage the foliage and the crop, were also noticed in plantations of large cardamom around Gangtok.

Minute grey spots with chlorotic haloes developed on the leaves mostly from the tip or margin. The spots were amphigenous and irregular in shape, with a prominent reddish-brown margin surrounded by chlorotic haloes. The spots were 130–200 mm × 26–45 mm in size, but under favourable conditions the entire leaf blade was blighted very fast. After some time, the central necrotic portion turned straw-coloured and appeared studded with numerous black dots on both the surfaces. Several such spots often coalesced to give a blighted appearance to the foliage. In the rainy season the necrotic tissues of the infected leaf withered away.

Repeated isolations from the infected leaves yielded a fungal pathogen, which was identified as *Pestalotiopsis versicolor* (Speg.) Steyaert. Colonies on potato dextrose agar are white, with aerial mycelium diffuse towards irregular advancing edge but denser towards the older part of the colony. Acervuli develop from yellowish clumps of hyphae and produce greenish-black spore masses. Diurnal zonations of mycelial growth and acervulus formation are apparent. No pigmentation or discoloration of the mycelium was visible on the reverse side. Conidia (21.5–27.5 × 4.35–8.75 μm) 4-septate, with 3 middle cells dark or brown and end cells hyaline. Apical cell bears 2 or 3 appendages while basal cell ends in a simple pedicel or a single basal appendage (figure 1).

Pathogenicity of the fungus was established by spraying spore and mycelial suspensions on healthy leaves. Humidity above 85% RH and temperature between 23 and 28°C favoured development of the disease. Typical disease symptoms appeared on both younger and older leaves 6–10 days after inoculation, but infection on younger leaves appeared early. The fungus has not been reported earlier on *Amomum subulatum*[1, 2]. Therefore this report constitutes a new host record for *P. versicolor* and also a new disease record for large cardamom. Earlier the fungus has been recorded on many hosts, including *Mangifera indica*, *Eugenia jambolana*, *Terminalia tomentosa*, *Typha angustata*, *Musa paradisica* var. *robusta*, *Jatropha curcas* and *Aloe vera*. The diseased specimen and culture have been deposited at the herbarium of CMI, Kew, England, under reference No. IMI 276700.

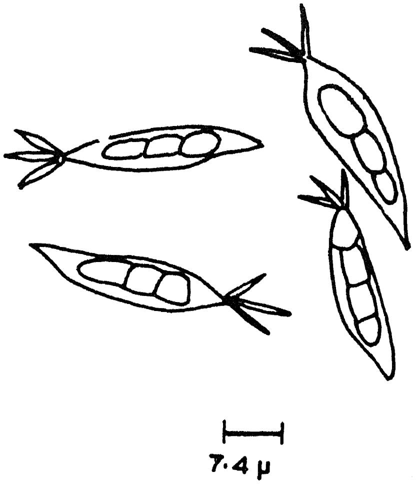

Figure 1. Camera-lucida diagram of conidia of *P. versicolor* (Speg.) Steyaert.

The authors thank the Director, CMI, Kew, England, for confirming the identification of the fungus.

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TRANSMISSION OF *RHIZOCTONIA SOLANI* (KUHN) IN SEEDS OF BEAN (*PHASEOLUS VULGARIS* L.)

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RHIZOCTONIA SOLANI (Kuhn) is pathogenic to bean, *Phaseolus vulgaris* L., causing web blight[1]. Hedgecock[2] observed mycelium and sclerotia of *R. solani* in the seed coat of bean. *R. solani* has so far not been reported to be associated with bean seed in Egypt. Hence its occurrence, location in different seed parts, and transmission through seed and soil were studied.

Seeds were collected from Alexandria, Beheira and Giza in Egypt. Incidence of seed infection was determined using the standard blotter paper method[3]. For location of the pathogen in different seed parts, seeds soaked in water were dehulled; seed coat, cotyledon and embryo axis were separated. The separated components were placed on water agar in petri plates and incubated according to ISTA[3] procedures. Highly infected seeds and seedlings were selected and histologically processed following the methods described earlier[4, 5]. To study transmission of the pathogen through seeds, a pot culture test[6] was conducted using sterilized soil.

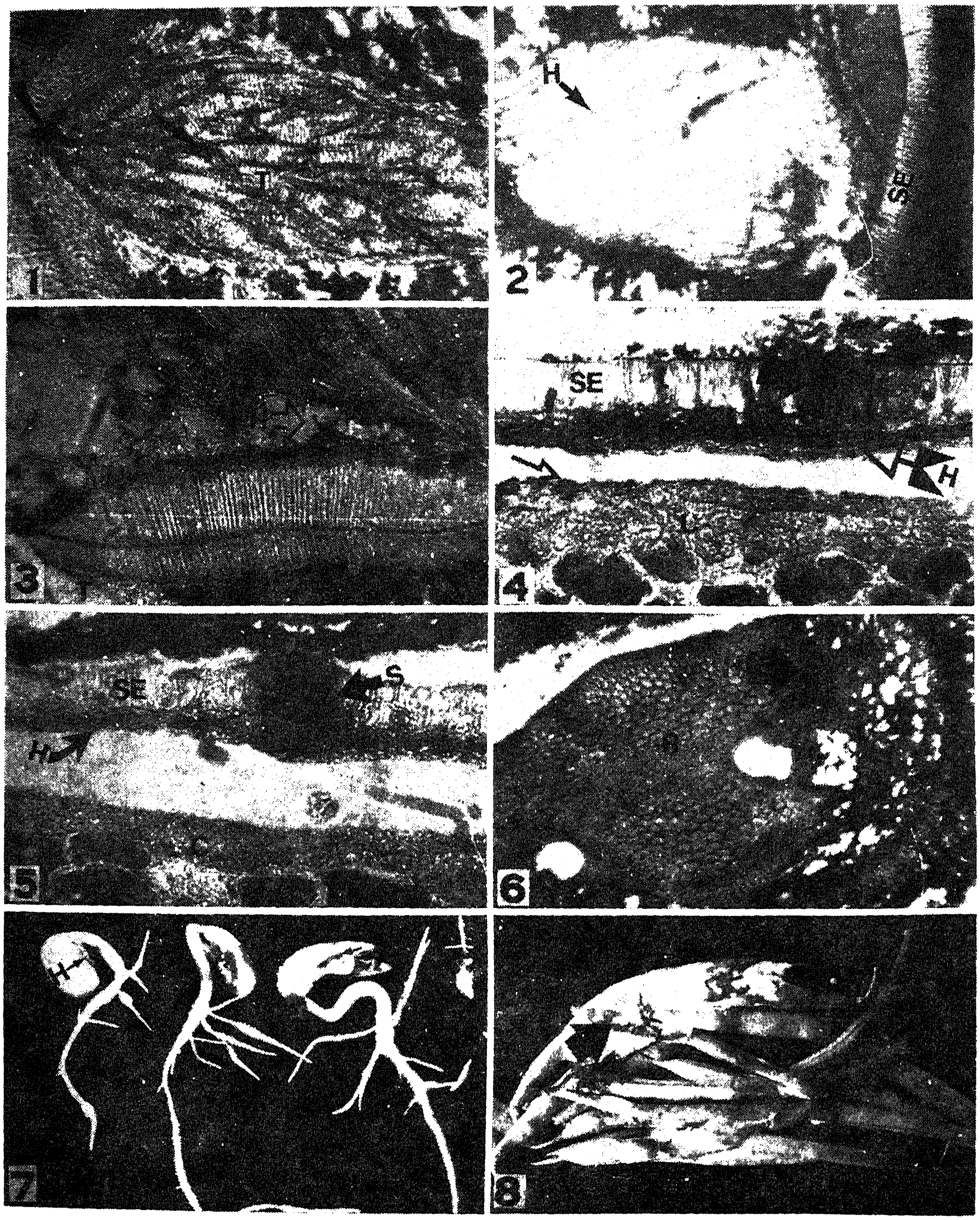
The pathogen was recovered in high percentage from seed coat and cotyledons, and in lower percentage from embryo axis. Histological studies indicated that seed penetration occurred mainly through the hilum tracheids (figure 1). Direct penetration through the intact seed coat was also observed in many seeds (figure 4).

The fungus colonizes the bean seed in all parts; hilum (figures 2 and 3) and cotyledon (figure 4) were the main sites of infection. Many reports indicate that the cotyledons are the main target of infection of many fungi that infect the embryo of legumes. Sclerotia were observed in the seed coat in some seeds (figure 5). In infected cotyledons, parenchyma cells of the outer part of the cotyledons were shrunken and devoid of cell contents (figures 4 and 5).

In 4-day-old seedlings from infected seeds fungal hyphae were observed on the seed coat, below the palisade layer, in the cotyledonary tissue, and in most tissues of the radicle (figure 6). Sclerotia were not observed deep in the tissues.

Transmission studies showed that infected seeds produced diseased seedlings, and 27% of the seedlings died within 4 days from germination.

Seed borne fungi



Figures 1–8. *Rhizoctonia solani* in seed of *Phaseolus vulgaris*. **1,** Fungal hyphae penetrating the seed through the hilum tracheids. **2,** Hilum tissues colonized by fungal mycelium. **3,** Extensive growth of mycelium in the hilum area. **4,** Cross-section of seed showing direct hyphal penetration through the seed coat. Hyphae can be seen located between the seed coat and the cotyledon and have invaded the outer parts of the cotyledon (arrow). **5,** Formation of sclerotia in the seed coat, with fungal mycelium between the seed coat and the cotyledon. **6,** Cross-section of infected seedling showing mycelium colonizing radicle tissues. **7,** Symptoms of infection in young seedlings. Note stunted growth of the seedling on the left due to the infection. **8,** Green bean pods, 24 h after storage, showing heavy fungal growth and pod rot. (C, Cotyledon; H, hyphae; R, radicle; S, sclerotia; SE, seed coat; T, tracheids.)

Symptoms on seedlings are sore shine, root rot, stem rot, necrotic leaves, dark pathces on cotyledons, and leaf spots (figure 7). Green pods had infection in many parts, and the hyphae colonizing most of the pods surfaced after 24 h of incubation in storage conditions (figure 8), causing pod rot.

Results also indicated that the pathogen was transferred from infected seeds to the soil. This means that infected seeds can cause spread of the pathogen in soil, where they are not known to occur. The danger of transmitting the pathogen through seeds has been reported earlier[12, 13].

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SELECTION FOR RED ROT RESISTANCE IN SUGARCANE

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RED rot is the most destructive disease of sugarcane in India. Many important varieties have gone out of cultivation because of this disease. Control methods are ineffective. Sugarcane being a highly heterozygous and polyploid species, one needs large progenies/seedlings for a search for disease-resistant varieties or clones. Degree of parental resistance had no effect on frequency of resistance in seedlings[1,2], but results contrary to this have also been reported[3,4]. The present study was therefore undertaken to study the role of parental resistance in the progenies of 14 intervarietal crosses.

Seven parental varieties, viz. the moderately resistant CP 44-101, Co 7637 and Co 1148; the moderately susceptible Co 7620 and Co 617; the susceptible Co 62174; and the highly susceptible Co 7717 were studied[5]. The fluff (true seed) of 14 crosses (table 1) was sown in a glasshouse in February 1982. The seedlings were transplanted in the field on 25th May 1982. Plant-to-plant and row-to-row distances were kept 75 and 90 cm respectively. Seedlings were harvested in February 1983 and ratoon crop was maintained. After six months standing canes were inoculated by the standard plug method[6]. The inoculated canes were split open longitudinally after 100 days and were graded into 5 grades (1, highly susceptible, to 5, resistant) on the basis of linear spread, condition of the top leaves, width of the lesion, and presence or absence of white spots, as suggested by Srinivasan and Bhat[7].

Table 1 shows the percentages of seedlings with the different grades of red rot resistance. Higher percentage ($\geqslant 20\%$) of resistant/moderately resistant seedlings was recorded in five crosses, all of which involve CP 44-101 as one of the parents.

Correlation between mid-parental reaction and percentage of resistant and moderately resistant clones among the progenies was positive and highly significant ($r = 0.55^*$ and 0.86^{**}), whereas correlation with percentage of susceptible and highly susceptible clones was negative and highly significant

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*For correspondence

Table 1 *Red rot rating and per cent selected clones from 14 crosses of sugarcane*

Cross (parental reaction)	Av. score	No. of clones	Per cent clones					Selected clones (%)
			R[a]	MR	MS	S	HS	
CP 44-101 × Co 1148 (MR × MR)	4.0	41	12	29	21	8	30	17.67
CP 44-101 × Co 617 (MR × MS)	3.5	59	2	22	24	12	40	3.38
CP 44-101 × Co 61174 (MR × S)	3.0	85	0	22	22	10	46	2.35
Co 7620 × CP 44-101 (MS × MR)	3.5	99	1	20	27	17	35	9.09
Co 7620 × Co 62174 (MS × S)	2.5	65	0	9	15	22	54	6.15
Co 7620 × 7717 (MS × HS)	2.0	48	0	4	10	19	67	2.08
Co 7637 × CP 44–101 (MR × MR)	4.0	31	0	20	23	9	48	9.67
Co 7637 × Co 62174 (MR × S)	3.0	26	0	12	11	27	50	3.85
Co 7637 × Co 7717 (MR × HS)	2.5	55	0	11	22	15	52	7.27
Co 1148 × Co 617 (MR × MS)	3.5	76	2	14	12	11	61	5.26
Co 1148 × Co 7717 (MR × HS)	2.5	19	0	10	21	16	53	0.00
Co 1148 × Co 62174 (MR × S)	3.0	40	0	12	20	15	53	5.00
Co 617 × Co 62174 (MS × S)	2.5	78	0	4	18	23	55	5.12
Co 617 × Co 7717 (MS × HS)	2.0	81	0	8	22	19	51	4.24
With Av. score } Correlation r_i			0.55*	0.86**	0.34	− 0.64**	− 0.65**	
With Av. score } Regression b_i			2.63	9.60	2.62	− 5.41	− 9.45	

[a] R, resistant; MR, moderately resistant; MS, moderately susceptible; S, Susceptible; HS, Highly susceptible. *·**, Significant at 5% and 1% respectively.

($r = -0.64$** and -0.65**). The trend in regression values was similar. These results show that resistant parents gave higher percentage of resistant seedlings and lower percentage of susceptible seedlings and vice versa. Such results can be exploited to select resistant varieties by incorporating resistant parents in the breeding programme.

Out of 803 seedlings 49 were selected on the basis of red rot resistance, general vigour, cane thickness, erectness and brix values in juice. The cross CP 44-101 × Co 1148 had the highest percentage of desirable seedlings (17.67). The results indicate the high general combining ability of CP 44-101 for red rot resistance, and CP 44-101 × Co 1148, Co 7637 × CP 44-101, Co 7620 × CP 44-101, and Co 7637 × Co 7717 were specific combinations for desirable clones with red rot resistance in sugarcane.

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ROLE OF SURFACE WAX OF CHILLI FRUITS IN INDUCING RESISTANCE TO FRUIT ROT PATHOGENS *COLLETOTRICHUM CAPSICI* AND *HELMINTHOSPORIUM ROSTRATUM*

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FRUIT rot of chilli (*Capsicum annuum*) is one of the important diseases that affects red ripe fruits. The pathogens *Colletotrichum capsici* and *Helminthosporium rostratum* were isolated from the majority of fruit samples showing fruit rot. The role of surface wax in the resistance of green fruits to fungal infection was studied. Surface wax was found to confer resistance to *Gloeosporium limetticola* in *Citrus aurantifolia*[1,2] and to *Erysiphe polygoni* f. sp. *hordei* in barley[3].

Healthy fruits of three different ages, viz. 5, 15 and 25 days, free from visible injury, and uncontaminated by spray chemicals were taken from plants. Surface wax was extracted from 5 g of fruit skin and estimated following the procedure of Martin and Batt[3]. The fruit skins were dipped in ether and gently agitated for 15–30 sec. This was repeated in four successive beakers containing ether. The ether extracts were made up to 25 ml. The volume of ether extract was reduced to 10 ml and the wax present in the ether extract was precipitated by adding excess of acetone. The solvents were then allowed to evaporate and the precipitate dried. The difference between the final and initial weight of the beakers gave the wax content of fruits. It was expressed as mg/g fresh weight and mg/g dry weight. Seven samples were used for each age group.

The surface wax extracted from the fruits was coated on a glass slide by a method slightly modified from Blakeman and Sztejnberg[5]. The wax (2 mg) from each fruit sample was dissolved in 10 ml of acetone and the solution was covered with a plastic cover. A wick of cotton thread was placed in contact with the wax solution. The other end of the wick was passed through a glass tube placed over the plastic cover and then allowed to touch a cavity slide. The wax solution rose through the wick and got deposited uniformly inside the cavity slide. This process was allowed for 48 h and the cavity slide was removed and placed in a ventilated container for seven days. To this cavity slide, 0.1 ml of spore suspension ($\sim 10^6$/ml) of *C. capsici* and *H. rostratum* containing the wetting agent Tween-20 was added. The slide was incubated in a moist chamber for 8 h and the number of germinating spores was counted. One hundred spores were examined per sample and four samples were used for the study. Spores placed in a drop of water served as control.

Entry of fungi into fruits of different ages was studied by the method of Shipton and Brown[6]. Chilli fruits of different ages, viz. 5, 15 and 25 days, were surface-sterilized with 0.1% mercuric chloride, washed repeatedly in sterile water, sprayed with spore suspension ($\sim 10^6$/ml), and incubated in a moist chamber for 48 h. The skin of the fruits was then cut into bits and immersed immediately in 10–15 ml of alcoholic cotton blue. They were boiled for 90 sec and then allowed to remain in the stain for 48 h at room temperature (28 ± 2°C). After rinsing with water, they were placed in chloral hydrate solution for 30 min, mounted in 50% glycerine, and examined under an oil immersion lens for conidial germination and entry.

Surface wax content of chilli fruits of different ages is presented in table 1. An interesting trend was noticed when the quantity of surface wax was expressed on the basis of two factors. While there was no significant difference between fruits of the three ages when wax content was expressed on fresh weight basis, it was highest in 5-day-old fruits when expressed on the basis of the dry weight of the fruits. In the experiment to study the possible role of wax in inhibiting spore germination, no significant difference was seen in the germination of spores in the presence of surface wax extracted from fruits of different ages (table 2). The tissue clearing technique revealed that the germination of spores and formation of appressoria were noted in fruits of all the ages. However, when sections were examined, entry of fungus was seen only in 25-day-old red ripe fruits.

Table 1 *Surface wax content of chilli fruits at different ages*

Age of fruits (days)	Surface max	
	(mg/g fresh weight)	(mg/g dry weight)
5	2.90	21.84
15	3.00	21.74
25	3.40	15.40
C.D. ($P = 0.05$)	NS	2.99

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*For correspondence

Table 2 *Effect of surface wax on germination of spores of Colletotrichum capsici and Helminthosporium rostratum*

Treatment	*C. capsici* (% germination)	*H. rostratum* (% germination)
5-Day old fruit wax	93.63 (80.02)	96.63 (79.73)
15-Day-old fruit wax	94.50 (76.73)	95.00 (77.77)
25-Day old fruit wax	96.25 (79.57)	95.75 (79.74)
Control (sterile water)	99.13 (87.27)	99.76 (88.57)

Figures in parentheses are transformed values.

	C.D. ($P=0.05$)
Fungus	NS
Treatment	4.55
Fungus × treatment	NS

In the present study, the amount of wax was higher in resistant green fruits than in susceptible ripe fruits, when expressed on dry weight basis. The chief components of cuticle are cutin and wax. Cutin forms the framework of the cuticular membrane which carries the wax on its surface and embedded within it[7]. A difference in quantity of wax between susceptible and resistant tissues has been reported in leaves of *Citrus aurantifolia* with reference to infection by *Gloeosporium limetticola*[1,2].

Dickinson[8] discussed the possibility that the waxy surface may present the first barrier by repelling the water film required by the pathogen for germination. Nutmen and Roberts[9] ascribed differences in susceptibility of coffee varieties to berry disease caused by *Colletotrichum coffeanum* to some physical and chemical differences in the cuticle, which made penetration of the resistant variety more difficult.

The possible mechanisms of interrupting infection are: (i) thickness of natural waxy layer in barley against *Erysiphe polygoni* f. sp. *hordei*[3], (ii) repelling of film of water on the leaf surface in sorghum against *Peronosclerospora sorghi*[10], and (iii) chemical substance in the cuticle of chrysanthemum against *Botrytis cinerca*[11]. After a critical examination of the role of wax in disease resistance, Royle[12] suggested that there was some evidence that cuticle provided protection against pathogens.

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MANGIFERINE-INDUCED CHROMOSOME ABERRATION IN ROOT-TIP CELLS OF *SOLANUM INCANUM* L.

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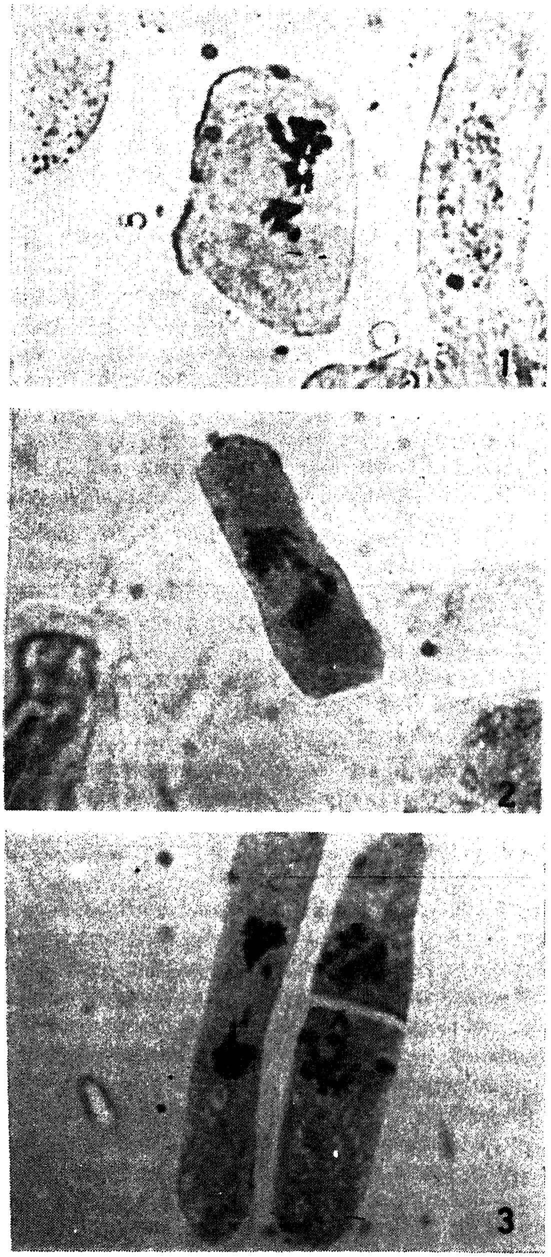
ROOT-TIPS from mangiferine-treated seeds of *Solanum incanum* showed several kinds of chromosome aberrations. The frequency of aberration increased with concentration of mangiferine. These observations are suggestive of its mutagenic property. The correlation coefficient between the aberrations and concentration is significant at the 1% level.

Mangiferine, a naturally occurring glucosylxanthone, is widely distributed in higher plants[1] such as in the members of the families Gentianaceae[2] and Anacardiaceae[3]. It has been found to work as an antimitotic and antifertility agent in animals, particularly mammals[4,5]. These effects had not been known in plants. If this drug can cause antifertility in plants, it may be of use in plant breeding programmes, specially in those cases where fruits and seeds are not required. The present paper reports some initial observations on mitotic chromosome aberrations induced by mangiferine treatment on dormant seeds.



Figures 1–3. Abnormal root-tip cells of mangiferine-treated seeds of *Solanum incanum* (×600). **1,** stickiness of chromosomes at metaphase; **2,** sticky bridge; **3,** lagging chromosomes at anaphase in one cell, and completed cytokinesis in the other.

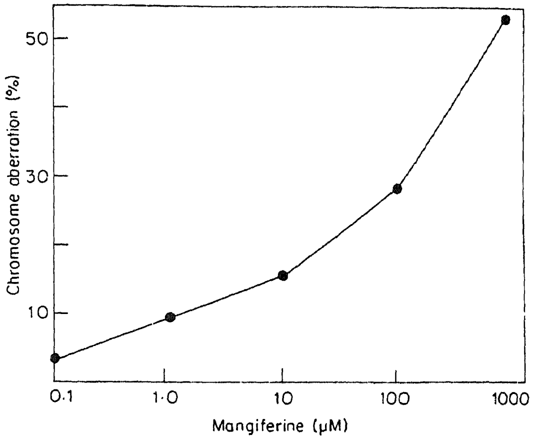


Figure 4. Frequency of chromosome aberrations in *Solanum incanum* with increasing concentration of mangiferine.

Genetically pure, healthy seeds of wild *S. incanum* were treated with different concentrations of mangiferine (molar concentration prepared in phosphate buffer, pH 7) ranging from 0.1 to 1000 μM for 6 h, washed with tap-water and grown in a sand-bed in a greenhouse. The emerging root tips were fixed in Carnoy's solution (acetic acid : alcohol, 1 : 3) for 24 h and squashed in 1% acetoorcein. About 300 root-tip cells were analysed for each kind of chromosomal abnormality.

Cytological analysis of root-tip cells revealed several kinds of chromosome anomalies such as stickiness of chromosomes, bridges, laggards or sticky bridges and fragments (figures 1–3). These abnormalities increased with concentration of mangiferine (figure 4). At 10 mM mangiferine caused necrosis at the root apex; shoots also turned pale and failed to survive. Lower concentrations (10 μM to 100 μM) also induced pale seedlings at low frequency but the seedlings later turned green. The correlation coefficient between concentration of mangiferine and induced chromosome aberrations was highly significant ($r = 0.901$). Thus the present observations suggest that mangiferine is a mutagenic chemical. Perhaps it interacts with nucleoprotein to cause damage to chromosomes. Chromatid bridges with lagging chromosomes appear owing to breakage and reunion of chromosomes[6]. Mangiferine may also prevent spindle formation.

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CALLUS INITIATION AND REGENERATION POTENTIAL IN DIFFERENT GENOTYPES OF *ERUCA SATIVA*

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ERUCA SATIVA Mill. 'taramira' ($n=11$, genome formula–EE) is highly resistant to aphids (*Lipaphis pseudobrassicae*) and thrives well under both rainfed and drought conditions[1]. Besides, its oil is rich in erucic acid[2], an industrially useful compound. Direct utilization of *E. sativa* in a breeding programme has been limited owing to the presence of strong incompatibility barriers with other *Brassica* species. Attempts are now being made to use *in vitro* techniques for the genetic amelioration of this crop species[3,4]. The present study was undertaken to identify genotypes responsive to *in vitro* culture and to develop regeneration protocols from cotyledonary explants of *E. sativa*.

Seeds of *E. sativa* cvs. T-27, TMH-46 and TMH-48 were surface-sterilized for 20 sec with 90% ethanol and for 7 min with $HgCl_2$ solution, washed thoroughly in sterilized distilled water, and germinated on agar (0.8%)/sucrose (2.0%) medium under a light and dark cycle of 16/8 h at 25 ± 1°C. After 7–8 days, the cotyledonary leaves were excised and cultured on MS[5] medium supplemented with cytokinins and/or auxins (table 1). About 100 cotyledons were cultured per medium/genotype tested. The number of explants regenerating shoots and the number of shoots per regenerating explant were recorded after 30 days of culture. All the cultures were kept under continuous fluorescent light of 5000 lux intensity at 25 ± 1°C.

Cotyledonary explants enlarged significantly within a week of culture and exhibited three kinds of response: callus, root formation and shoot formation (table 1). Callusing was observed at the cut ends of the explants in all the cultivars and on all the media, although with different efficiencies. Callus proliferation could be increased by causing multiple injuries

Table 1 *Effect of different media on callus induction and root and shoot regeneration in cotyledonary explants of different cultivars of Eruca sativa*

		Cotyledons (%) forming		
Medium	Cultivar	calli	roots	shoots
MS + 0.05 mg/l NAA	T-27	64.6	89.6	2.1 (0–1)*
	TMH-46	57.8	85.6	1.1 (0–1)
	TMH-48	60.0	90.8	1.5 (0–1)
MS + 0.5 mg/l NAA + 2 mg/l BAP	T-27	87.1	77.0	20.7 (0–10)
	TMH-46	90.0	84.5	15.6 (0–10)
	TMH-48	93.3	85.0	8.3 (0–5)
MS + 0.2 mg/l NAA + 2.0 mg/l kinetin	T-27	95.8	91.5	15.3 (0–10)
	TMH-46	93.1	85.4	10.4 (0–5)
	TMH-48	91.4	81.4	12.9 (0–6)
MS + 2 mg/l NAA + 10 mg/l kinetin	T-27	3.9	11.8	1.0 (0–1)
	TMH-46	10.7	14.6	1.0 (0–1)
	TMH-48	33.4	36.2	1.0 (0–1)

*Number of shoots per regenerating explant.

Tissue culture

to the cotyledonary leaves. Callus initiation and its further growth was maximum on MS medium supplemented with either kinetin (2 mg/l) or benzylaminopurine (BAP, 2 mg/l) and a low concentration of naphthaleneacetic acid (NAA). Kinetin and NAA at higher concentration caused little or no callus formation. NAA alone could support moderate callus growth only.

Winthin 2–3 weeks, the callus started differentiating into nodular structures and formed shoot buds, which subsequently gave rise to multiple shoots. Frequency of cotyledons responding to plant regeneration depended upon the plant genotype and the hormonal constitution of the medium (table 1). MS medium supplemented with BAP and NAA was most effective and gave highest frequency of shoot regeneration. The frequency of shoot regeneration declined when BAP was substituted with kinetin. Media containing kinetin and NAA together at high concentration or NAA alone gave poor regeneration frequencies.

When 10–15-day-old regenerated shoots were separated and subcultured individually onto fresh medium, multiple shoots (1–25) could be seen regenerating from the callus formed at the base of the transferred shoot. In one case as many as 98 multiple shoots could be obtained from a single cotyledon explant in two subcultures.

There were significant differences in the shoot regeneration response of the three cultivars of *E. sativa*. The best regeneration was observed in cv. T-27, which gave an average of 9.8% explant cultures with shoots. TMH-46 and TMH-48 gave 7% and 5.9% explant cultures with shoots respectively.

Most of the regenerated shoots developed roots in the parent medium. The remaining shoots could be easily rooted when cultured on MS medium supplemented with NAA, after giving a cut at the base.

The study showed that genotype of the plant and hormonal constitution of the medium determine the shoot regeneration response of explant tissue in *E. sativa*. A proper balance of cytokinin and auxin is necessary to achieve high regeneration frequencies. The procedure and the mode of plant regeneration are similar to that reported for other *Brassica* species[6, 7]. By using such techniques it may be possible to select genetically modified *E. sativa* through *in vitro* culture and to develop agronomically improved strains of this crop.

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THIN-LAYER CHROMATOGRAPHY ON CELLULOSE

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THIN-LAYER chromatography (TLC)[1,2] is a rapid, simple and versatile method for separation of mixtures of phytochemicals because of its speed and sensitivity as well as the availability of a variety of adsorbents like silica gel, alumina, celite, calcium hydroxide, magnesium phosphate, polyamide, Sephadex, polyvinylpyrrolidone and cellulose, or a mixture of two or more of them.

Ready-to-use TLC plates are not yet common in Indian laboratories, especially plates coated with silica gel, cellulose and polyamide. The preparation of TLC plates coated with silica gel[3] is comparatively easy but their preservation is difficult; on the other hand the preparation of cellulose plates[4] suited for a specific purpose is difficult though the prepared plates are comparatively easily preserved and handled. Under these circumstances and in view of the fact that cellulose TLC shows much higher resolution and speed than PC, it was decided to develop and test cellulose plates for qualitative and preparative analysis. The present study relates to the preparation of cellulose TLC plates and examination

Polyphenolics and Sugars

Table 1 *Cellulose TLC of plant constituents*

Compounds	Solvent composition	Detection
Phenol and phenolic acids	Benzene–methanol–water (45:8:4)	Vanillin–conc. HCl
Phenylpropanoids	Aceticacid (15%) Benzene–aceticacid–water (6:7:3) Water (15%)	UV/UV–ammonia
Flavones and flavonols	Aceticacid–conc. HCl–aceticacid–water (3:30:10) Phenol (water-saturated) Water	UV/UV–ammonia
	n-Butanol–aceticacid–water (3:1:1)	UV/diphenylborinate
Anthocyanins	Amyl alcohol–aceticacid–water (2:1:1) Formicacid–conc. HCl–water (10:1:3)	Visible/UV/UV–ammonia
Chalcones and aurones	Isopropanol–acetone–water (5:1:4) *n*-Propanol–aceticacid–water (1:1:1)	Visible/UV/UV–ammonia
Sugars (monosaccharides)	Benzene–*n*-butanol–pyridine–water (5:1:3:3) *n*-Butanol–aceticacid–water (4:1:5, upper) *n*-Butanol–ethanol–water (4:1:5) Phenol	Aniline–hydrogen–phthalate

of their suitability for the separation of phytochemicals.

Two types of cellulose adsorbents, viz. microcrystalline cellulose for TLC (Loba-Chemie Indoaustranal Co., Bombay, India) (A), and cellulose powder adsorbent for TLC (Biochemicals Unit, University of Delhi, India) (B), were used.

The technique of preparing cellulose chromoplates involves the mixing of 20 g of A in 140 ml of water and homogenization in a mechanical mixer for 3–5 min. Coating is done using standard TLC applicators, with film thickness adjusted to 0.5 mm. The plate was gently rocked until the material was evenly distributed over the surface. After air-drying (28–30°C) for about 5 h the plate was ready for use. No binder was needed because of the particulate nature of cellulose powder. Care should be taken to eliminate air bubbles while transferring adsorbent slurry to the applicator. Plates were prepared in the same manner for the cellulose sample B, except that the slurry was prepared by mixing 20 g of adsorbent in 100 ml of water.

Fifteen plates (20 × 5 × 0.05 cm) could be prepared in the case of cellulose powder A and 12 chromoplates in the case of cellulose powder B with the quantities mentioned. For two-dimensional separation, square chromoplates (20 × 20 × 0.05 cm) were found suitable. Of the two samples A and B, A was observed to be superior with regard to compactness of the spot and resolution.

Separation into homogeneous components from mixtures of hydrophilic plant constituents was achieved using cellulose plates made as described and using the developing solvents indicated in table 1.

Preliminary investigations were performed for hydrophilic plant products. Development was accomplished with various solvent mixtures prepared from analytical grade reagents. The most suitable ones for the common plant constituents (especially phenolic compounds and sugars) are given in table 1.

Our study confirmed the general observation of the superiority of cellulose in TLC separation. It has an edge over the seemingly similar technique of silica gel TLC in the following aspects: the plates can be safely handled and preserved, the resolution is superior, and the eluate after centrifugation is generally free from the adsorbent. Mixtures of flavonoids containing up to nine constituents could be separated by a one-stage 2D–TLC over cellulose whereas 2D silica gel TLC as well as 2D–PC fails to achieve this. Details of the compounds isolated and identified from plant extracts will be published elsewhere.

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ESTIMATION OF QUERCETIN IN THE LEAVES OF *MAESA INDICA* WALL BY A NEW COLORIMETRIC METHOD

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THE leaves of *Maesa indica* are used in curries in North India and also as fish poison. Extracts of the branchlets, leaves, bark and stem have insecticidal activity[1]. Locally the plant is called 'vavding'. It is used as a blood purifier and as an anthelminthic by the local people. This communication reports isolation and estimation of quercetin from the leaves *M. indica* from Goa.

The methods reported in the literature for quercetin estimation are Folin–Ciocalteau spectrophotometry[2-5], fluorimetry[6], colorimetry by using Folin–Ciocalteau reagent[7], and by treating with NaOAc, HOAc (3:1) and $AlCl_3$ (ref. 8). In the present work a simple and sensitive method that gives very stable colour for more than one hour is described. In the Folin–Ciocalteau method the authors described the method for general phenolic substances from apple juice, while the present method is specific for quercetin only. In the NaOAc, HOAc and $AlCl_3$ method the colour obtained is stable for 20 min whereas in the present method the colour is stable for more than one hour. The present method is more sensitive than the earlier methods, and is good up to 2 μg quercetin.

Mature leaves of *M. indica* were collected at the beginning of winter from the Morlem forests of Sattari taluka in Goa. A fruiting voucher specimen of the plant is preserved in the phytochemistry research lab., Goa College of Pharmacy. The leaves were washed in running tap-water, air-dried and powdered. The leaf powder (100 g) was defatted with petroleum ether. The marc was then extracted with methanol, and the extract reduced to 50 ml and mixed with 6% HCl (50 ml). The solution was heated on a water bath for 45 min. The hydrolysate was extracted thoroughly with ether in a separating funnel. On separation the ether layer was concentrated to residue[9]. The residue was digested with H_2O, filtered and then dissolved in *n*-propanol. It was further purified by preparative TLC (silica gel G, using EtOAc: HCOOH:AcOH:H_2O, 100:11:11:27)[10,11]. The compound obtained was recrystallized from methanol and was confirmed from UV and IR spectra as quercetin.

A new colorimetric method was developed for quantitative analysis of quercetin by reacting it with acidic ammonium molybdate in *n*-propanol medium using Speckol Spectrocolorimeter (Carl Zeiss Jena).

Quercetin (10 mg) was dissolved in 100 ml of *n*-propanol to obtain a reference solution. 10 g of leaf powder was defatted with petroleum ether and thoroughly extracted with methanol in a continuous hot extractor. The methanolic extract was filtered and concentrated to 5 ml. This concentrate was subjected to acid hydrolysis as in the isolation procedure and the volume was made up to 50 ml. Two ml of this was made up to 100 ml with *n*-propanol to obtain a test solution.

Three activated silica gel G plates were streaked with 0.25, 0.5 and 0.75 ml of the reference and test solutions. The solvent system used was using EtOAc:HCOOH:AcOH:H_2O (100:11:11:27). Scrapings of the band corresponding to quercetin were transferred to a stoppered test tube and extracted vigorously with 5 ml of *n*-propanol. After centrifuging the supernatant liquid was filtered through Whatman No. 42 filter paper into a 10 ml volumetric flask. To the above filtrate, 0.5 ml of 0.1 N H_2SO_4 and 1 ml of 10% (w/v) aqueous ammonium molybdate were added. The resulting yellow chromogen was measured at 420 nm. A graph of absorbance versus concentration was plotted and was found to be linear over the concentration range 2 to 14 μg/ml, indicating conformity with Beer's law.

The quercetin content of the leaves of *M. indica* was found to be 3.03%. Quercetin has also been reported in *Maesa macrophylla*[12].

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CATION EXCHANGE CAPACITY OF SOIL AND ADSORPTION OF *AZOSPIRILLUM* TO SOIL PARTICLES

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AZOSPIRILLUM occurs as a predominant diazotroph in the rhizosphere of grasses and cereals[1]. The population of *Azospirillum* in soil and the rhizosphere zone of plants is influenced by many factors, of which soil is an important one. Microorganisms in soil mostly occur adsorbed to soil particles and rarely as unadsorbed cells[2]. The influence of the cation exchange capacity (CEC) of soil on the adsorption of *Azospirillum* and other heterotrophic bacteria on soil particles is reported.

Three different soil types were used in these studies. Soil samples were collected from the top 0–10 cm, air-dried, and pounded and sieved through a 50 mesh sieve. The total heterotrophic bacteria in the soils were enumerated by the dilution plate technique on yeast extract glucose agar[3]. CEC of the soils was determined as described earlier[4]. One hundred grams of each soil was taken in a beaker and leached thrice with 1 N ammonium acetate, pH 7.0. A standardized cell suspension of *Azospirillum brasilense* strain S.3, which is resistant to both streptomycin and chloramphenicol at $100\,\mu g\,ml^{-1}$ of each and contains a pink chromogen (obtained from D. Hubbell, University of Florida, USA), was added to the soil samples and mixed well. Three replications were maintained in each treatment.

After overnight incubation, the populations of the inoculated *Azospirillum* and the total heterotrophic bacteria were estimated using yeast extract glucose agar. The soil samples were then leached thrice with sterile distilled water with minimum disturbance to the soil, after which the populations of the inoculated *Azospirillum* and total heterotrophs in the soil samples were again determined. As strain S.3 was pink the colonies of *Azospirillum* on the agar medium were easily counted. The percentages of inoculated *Azospirillum* and heterotrophs retained in the soil samples were calculated.

Some physical properties of the three soil types are presented in table 1. Adsorption of *Azospirillum* and heterotrophic bacteria on soil particles is given in table 2. The study revealed that the CEC of soil is closely associated with the adsorption of not only cells of *Azospirillum* but also of the heterotrophic bacteria of the soil. The higher the soil CEC, higher was the percentage of adsorbed cells. Alluvial (clay) soil with a CEC of 33.9 m.e. retained 82.8% of *Azospirillum* in adsorbed state. The cations present in the soils might have contributed to the increase in the adsorption of *Azospirillum* cells to soil particles. Calcium ion-mediated adsorption has been suggested for root nodule bacteria[5]. It is believed that when microorganisms are adsorbed to solid surfaces they enjoy nutritional advantages. Cells adsorbed to surfaces in water take up substances in solution more easily than unadsorbed cells which are likely to be carried away. Other advantages to the bacteria

Table 1 *Some physical and chemical properties of the soils examined in this study*

Soil type	pH	Total nitrogen (%)	Organic carbon (%)	Electrical conductivity (mmho × cm^{-1})	CEC (m.e./100 g/ of soil)
Alluvial soil (clay)	8.5	0.12	0.68	0.37	33.9
Laterite soil	6.0	0.10	0.60	0.09	19.3
Red loam	7.1	0.08	0.84	0.10	15.4

Table 2 *Adsorption of Azospirillum and heterotrophic bacteria to soil particles*

	Azospirillum (× 10^6)			Heterotrophic bacteria (× 10^6)		
Soil type	Initial	Final	Adsorbed cells (%)	Initial	Final	Adsorbed cells (%)
Alluvial soil (clay)	21	17.4	82.8	36.0	29.6	82.2
Laterite soil	21	12.0	57.1	28.8	18.4	63.8
Red loam	21	8.2	39.0	17.0	6.8	40.0

Bacterial numbers are per gram of soil on oven dry weight basis. Each figure is mean of three estimations.

are protection and proximity to a suitable environment[6].

28 July 1988

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MULTIPLE XANTHINE DEHYDROGENASE ISOZYMES IN THE EXOTIC CARP *CTENOPHARYNGODON IDELLA* (CYPRINIDAE: PISCES)

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XANTHINE dehydrogenase (XDH; E.C. 1.2.3.2) is a polymorphic enzyme involved in purine metabolism[1]. From the available data on XDH isozymes in fish[2–3], two codominant alleles at an autosomal locus are believed to be involved. XDH activity is known to respond to nutritional status of the organism[1] and is independent of age and sex[3]. Usually, homozygotes show one XDH band on electrophoresis, and heterozygotes three bands, suggesting dimeric structure of the enzyme[3]. So far as the authors are aware, XDH isozyme patterns in fish have not yet been reported from India. During our studies on the XDH isozyme patterns of some fishes, we came across multiple XDH isozymes in the exotic carp, *Ctenopharyngodon idella*.

Ten adult living specimens of *C. idella*, collected from the local fish farm (Ganga Matsya Utpadan Kendra, Rathtala), served as the material for the present study. Muscle, heart, liver, eye, kidney and brain were dissected out quickly and homogenized separately in cold (4°C) distilled water. The homogenate was centrifuged at 11,000 *g* at 4°C for 30 min. Known amount of supernatant was immediately subjected to polyacrylamide slab gel electrophoresis[4,5] at a constant current of 3 mA/slot and 200 V in the cold (6–7°C) using Tris–glycine (pH 8.6) as the running buffer. Staining for detection of XDH activity was done following Nakano and Whiteley[6].

Four or five XDH bands were observed in the zymograms (figure 1A, B) of all tissues examined, except liver, which showed two bands. Except for the slowly anodal band in kidney which showed intense

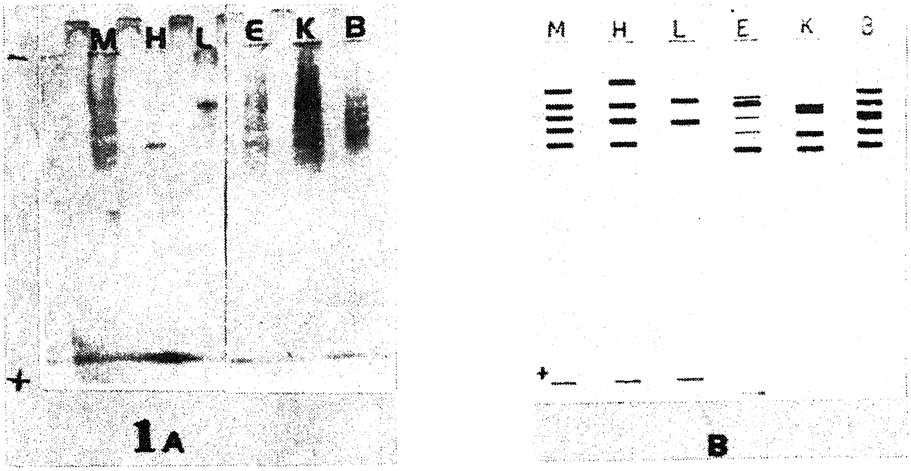

Figure 1. (A) Photograph and (B) diagrammatic representation of XDH zymogram of *C. idella* (M, muscle; H, heart; L, liver; E, eye; K, kidney; B, brain).

Table 1 *Number of XDH bands in zymograms of tissues of Ctenopharyngodon idella, their Rd values and intensity of staining*

Tissue	No. of bands	Rd values	Staining intensity
Muscle	5	0.146	1+
		0.191	1+
		0.224	1+
		0.258	1+
		0.303	1+
Heart	4	0.123	1+
		0.196	1+
		0.235	1+
		0.303	2+
Liver	2	0.179	2+
		0.241	1+
Eye	5	0.155	1+
		0.182	1+
		0.215	1+
		0.258	1+
		0.311	1+
Kidney	4	0.204	3+
		0.225	1+
		0.268	1+
		0.311	1+
Brain	5	0.150	1+
		0.182	1+
		0.220	1+
		0.258	1+
		0.301	1+

1+, Faint (marginal presence); 2+, moderate (intermediate abundance); 3+, intense (most abundant).

staining, all the bands were faintly or moderately stained. In all the tissues, the anodal migrations of the bands were more or less similar although the migration of individual bands differed slightly in different tissues (table 1).

In view of the dimeric structure of XDH, the expression of which is believed to be controlled by two codominant alleles at an autosomal locus[3], it is difficult to understand and explain the expression of 4 to 5 electrophoretically separable components in several tissues of *C. idella*. It may, however, be possible that conformational changes of the isozymes owing to physical/physiological factors could be responsible for the appearance of the extra bands in some tissues, as was also speculated by Shinoda and Glassman[7] in *Drosophila* and Waud and Rajagopalan[8] in a similar situation in mice. Alternatively, epigenetic modification during the synthesis of the isozymes in different tissues could also lead to the appearance of the unorthodox XDH patterns in this species. Another possibility, though remote, could be that the multiple bands represent activity of two homologous loci which might have arisen by duplication. Anyhow a detailed study on different populations, kinetic properties of the isozymes, amino acid sequence analysis, etc. are warranted to understand the genetic mechanism for the appearance of the multiple XDH bands in this species more precisely.

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STUDIES ON ELECTROPHORETIC PATTERN OF SERUM PROTEINS OF THE TOAD *BUFO ANDERSONII*

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AMPHIBIANS are cold-blooded vertebrates and are known to hibernate and aestivate during winter and summer seasons respectively. Atmospheric physical factors influence the physiology of the animals. Therefore it was proposed to study the serum protein fractions of toad by agar gel electrophoresis during winter and summer seasons.

Ten male and ten female toads were procured during winter and summer months. The animals were killed and blood was collected immediately from the heart into sterile tubes for serum separation. The serum was assayed for various protein fractions by agar gel electrophoresis[1] using barbiturate buffer (pH 8.6). The gels were stained with amido black and scanned on a densitometer.

The serum showed four distinct fractions, viz. albumin and alpha-, beta- and gamma-globulins. The proportions of these fractions are shown in table 1.

All the four serum protein fractions of toad were cathodic (figure 1). In fish[2], sheep[3], and buffalo[4] gamma-globulin is anodic.

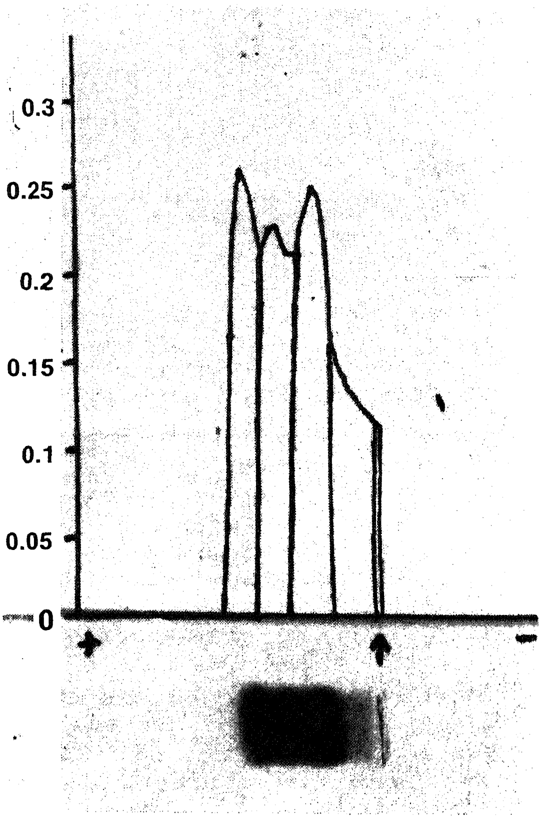

Figure 1. Agar gel electrophoretic pattern of serum of *Bufo andersonii*.

Table 1 *Proportions of various serum protein fractions of toad*

Sex	Season	Protein fraction*				Albumin/ globulin ratio
		Albumin	Alpha-globulin	Beta-globulin	Gamma-globulin	
Male	Summer	23.89 ± 0.715	25.51 ± 0.598	32.44 ± 0.674	18.14 ± 0.493	0.31
(n = 10)	Winter	23.27 ± 0.626	25.00 ± 0.556	32.75 ± 0.810	18.98 ± 0.515	0.30
Female	Summer	24.17 ± 0.462	21.38 ± 1.031	34.37 ± 0.651	20.06 ± 1.08	0.31
(n = 10)	Winter	24.09 ± 0.382	28.91 ± 0.993	31.72 ± 0.835	15.26 ± 1.189	0.31

*Values expressed as mean ± S.E.

Albumin/globulin ratio was nearly the same in all the cases. Beta-globulin fraction is the highest in all toads in both the seasons. This is similar to the findings reported earlier[5,6] for other animals. Gamma-globulin was lowest in all the cases.

The levels of alpha-globulin in male and female toads were significantly different ($P > 0.01$) in both seasons. Changes in globulin fractions were seen in female toads: beta- and gamma- globulin fractions increased significantly ($P > 0.05$ and $P > 0.01$ respectively) in summer. This is similar to the results of Ashton[7] for sheep. The alpha-globulin fraction was significantly higher during winter in female toads ($P > 0.001$). Perk and Loble[8] had found a direct genetic control on plasma protein fractions.

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RADIOMODIFYING EFFECT OF CHAMPHOR AS REVEALED BY SISTER CHROMATID EXCHANGE

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SISTER chromatid exchange (SCE) represents reciprocal exchanges between replicated sister chromatids at homologous points and its analysis is a sensitive means of monitoring genotoxicity[1-3]. Camphor has been reported to enhance radiation-induced damage in bacterial cells under hypoxic conditions[4], in mouse transplantable mammary adenocarcinoma[5], and in spermatogonia of adult mice[6]. In view of the potential utility of camphor in cancer therapy, studies were undertaken to evaluate the radiomodifying effect of camphor on normal tissues by SCE analysis. In this paper we report the radioprotective effect of camphor in mouse bone marrow.

Three-to-four-month-old inbred Swiss albino mice were randomly assorted into 15 groups (table 1), each with four animals. Each animal, except controls, received camphor (0.5 μmol/g body wt) dissolved in olive oil intraperitoneally. Whole-body gamma irradiation was given 30 or 45 min after administration of camphor. One day before sacrificing the animals, 9 injections of BrdU (10^{-2} M), spaced hourly, were administered intraperitoneally. Each injection contained deoxycytidine (5×10^{-3} M) to reduce the toxicity to BrdU. Seventeen hours after the last BrdU injection, colchicine (0.4 mg/ml) in distilled water was injected intraperitoneally (4 mg/100 g body wt). Two hours later, animals were sacrificed by cervical dislocation. Chromosome preparations were made and stained with Hoechst 33258 and Giemsa[7]. Animals were kept at 25 ± 2°C and food and water were given *ad libitum*.

Table 1 presents the frequency of SCE in bone marrow cells after various doses of gamma irradiation in the presence or absence of camphor. In the different control groups (first three rows in table 1) the frequency of SCE was within normal limits. Animals given camphor and no radiation showed enhancement in SCE frequency but that was not statistically significant. Animals exposed to increasing doses of whole body gamma radiation showed significantly higher incidence of SCE.

Animals administered camphor prior to irradiation showed a marked reduction in SCE compared to the correspondingly irradiated group that did not receive camphor (table 1 and figure 1). Compared to 30 min interval between camphor treatment and irradiation, 45 min interval resulted in a slight decrease in number of SCE. The difference between the 30 min and 45 min groups is significant only at 2.0 Gy radiation dose. Further, the reduction in SCE frequency due to camphor became more pronounced with increasing levels of radiation exposure. Maximum reduction in frequency of SCE by camphor was at 2.0 Gy. The radioprotective potential of camphor is thus evident from this study.

The bone marrow cells are well oxygenated and oxygen is an effective radiation sensitizer. The present findings agree with previous reports[1]. Goel *et al.*[6] studied radiosensitizing effects of camphor in the testicular system, which is often used as a hypoxic model for *in vivo* studies. They observed

Table 1 *Radiomodifying effect of camphor as assessed by effect on frequency of sister chromatid exhanges in bone marrow cells of Swiss albino mice*

Treatment	No. of metaphases scored	Total no. of SCEs	SCEs per metaphase Mean ± SE
Control (no treatment)	180	497	2.76 ± 0.08
Olive oil only	189	535	2.89 ± 0.07
Camphor + olive oil (0.5 μmol/g body wt)	250	866	3.40 ± 0.04
0.25 Gy only	300	1490	4.73 ± 0.06
0.25 Gy + camphor (30 min)	150	597	3.98 ± 0.06
0.25 Gy + camphor (45 min)	155	589	3.83 ± 0.06
0.50 Gy only	199	1118	5.61 ± 0.06
0.50 Gy + camphor (30 min)	222	926	4.23 ± 0.05
0.50 Gy + camphor (45 min)	190	792	4.20 ± 0.05
1.00 Gy only	165	960	5.85 ± 0.06
1.00 Gy + camphor (30 min)	170	767	4.65 ± 0.06
1.00 Gy + camphor (45 min)	145	618	4.19 ± 0.07
2.00 Gy only	5*	36	7.20 ± 0.65
2.00 Gy + camphor (30 min)	50	247	4.94 ± 0.12
2.00 Gy + camphor (45 min)	58	249	4.29 ± 0.10

*The low number is due to nonavailability of sufficient second division metaphases.

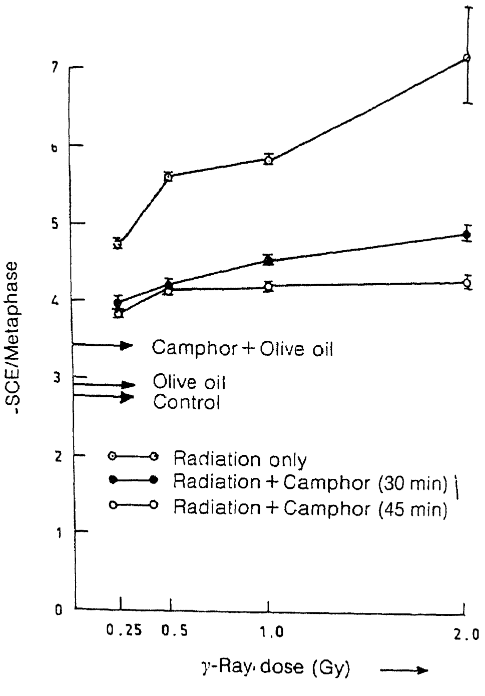

Figure 1. Effect of camphor on gamma irradiation-induced SCE in bone marrow cells of Swiss albino mice.

accelerated damage to resting primary spermatocytes in mouse testis due to irradiation in presence of camphor. Camphor has been shown to act as hypoxic sensitizer in bacterial cells[4] and also in mammary adenocarcinoma in C_3H Jax mice[5].

The radioprotective and radiosensitizing behaviour of camphor for euoxic and hypoxic cells respectively during irradiation show its potential for meeting the most ideal requirement of a chemical radiomodifier for improving clinical radiotherapy. However, the differential behaviour of camphor for oxic and hypoxic tissues is yet not well understood and further investigations in this direction are in progress.

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A NOVEL METHOD FOR CLONING CHROMOSOMAL DNA IN *HAEMOPHILUS INFLUENZAE* Rd

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PLASMID pRSFO885, genetically marked with an ampicillin-resistance marker[1], transforms *Haemophilus influenzae* very poorly[2]. However, if sequences homologous to chromosomal DNA are spliced to its only *Pvu*II site, transformation frequency increases a thousand-fold for the *amp*^{r} marker[3]. The enzyme *Pvu*II produces blunt ends in the DNA. Consequently, ligation to DNA segments is not as efficient as with a sticky-end-cut DNA vector and insert. A vector, pJ1-8, has been derived from pD7 (consisting of a chromosomal DNA segment spliced to pRSFO885) which has only one *Eco*R1 site (and no *Pvu*II site)[4]. Like pRSFO885, pJ1-8 gives little or no transformation for the *amp*^{r} marker, but when *Eco*R1-generated segments of chromosomal DNA are spliced to it, the frequency of Amp^R transformants increases by 3–4 orders of magnitude. Using this plasmid, several DNA clones carrying alleles of *nov*, *str*, *nal* and *uvr1* genes have been cloned[5–7]. However, most of the clones (except pKuvr1) are lost in the absence of selection pressure. A similar observation has been made[8] with DNA fragments cloned in pRSFO885. A new type of cloning system was serendipitously discovered. Plasmid pRSFO885 has two *Bam*HI sites, generating fragments designated here as A and B. The *amp*^{r} marker is on fragment A. In an attempt to clone DNA in one of the two sites by partial digestion, a few stable clones were obtained, although at a very low frequency. Analysis of some of these revealed that all such clones should carry a duplication of the 2.45 kb *Bam*HI fragment of pRSFO885. This report describes the construction and isolation of such clones.

A wild-type strain of *H. influenzae* Rd was used. Plasmids used were pJ1-8 (ref. 4), pRSFO885 (ref. 1), pJ1-8Str^{R}38, pJ1-8Str^{R}14 and pJ1-8Nal^{R}33 (ref. 6).

H. influenzae cells were grown at 37°C with aeration in BHI (brain heart infusion, Difco) broth containing 2 μg/ml NAD and 10 μg/ml haemin. Cells were grown to exponential phase, before freezing at −73°C in the presence of 15–20% glycerol.

Cells were made competent by the aerobic–anaerobic method[9]. A transformation mixture normally consisted of 0.1 ml competent cells + 0.1 ml DNA + 0.8 ml BHI broth. DNA uptake was allowed for 10–15 min before plating. Plates were challenged with ampicillin (5 μg/ml) after 1.5–2 h of incubation by overlaying with 10 ml BHI agar containing ampicillin.

Chromosomal DNA was extracted by the method of Marmur[10]. Plasmid DNA was extracted by the method of Hirt[11], with minor modifications. Plasmid DNA was purified by cesium chloride–ethidium bromide equilibrium density gradient centrifugation[12].

For electrophoresis of DNA, 0.7–1.4% agarose gels were used in Tris–acetate buffer (pH 8). Electrophoresis was carried out at 4 V/cm for 4–5 h[13].

Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories and used according to their instructions.

Plasmid loss was measured[8] by growing the cells overnight without ampicillin, and then plating on plates without ampicillin. Colonies were replica-plated on plates with or without ampicillin. Colonies that grew on plates without ampicillin but failed to grow on plates with ampicillin were considered to be those of cured cells.

pRSFO885 DNA has two *Bam*HI sites and it was considered possible that either of the two sites may be used for the cloning of DNA. Accordingly, *Bam*HI-digested chromosomal DNA was spliced with a partial digest of pRSFO885. Upon exposing a competent wild-type cell culture to the ligated DNA, a large number of Amp^R transformants were obtained (approximately 2×10^4 per ml). Five hundred Amp^R colonies were picked and analysed. Transformation using clear lysates obtained from these clones revealed that most of them give little or no transformation for the *amp*^{r} marker (less than 1×10^3 per ml) when reused for transformation. Only four clones were exceptions in that they gave high transformation frequency (table 1). These clones were designated pS43, pS121, pS322 and pS436. Agarose gel electrophoresis of the clear lysates showed that all the plasmids that fail to give *amp*^{r} transformation fall into any of the 3–4 groups of particular sizes (data not shown). Apparently,

Table 1 *Amp^R transformants obtained in Haemophilus influenzae Rd with various chimeric plasmids constructed using pRSFO885 for cloning at either BamHI site*

Plasmid	Amp^R transformants per ml ($\times 10^4$)
pS43	35
pS121	170
pS322	57
pS436	40

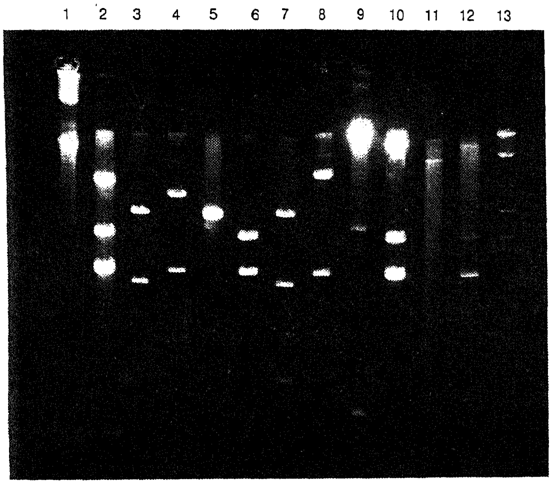

Figure 1. Agarose gel electrophoresis of restriction digests of various plasmids. Lane 1, pS121 digested with *Eco*RI; lane 2, pS121 digested with *Bam*HI; lane 3, pJ1–8Str^{R}14 digested with *Eco*RI; lane 4, pJ1–8Str^{R}14 digested with *Bam*HI; lane 5, pJ1–8 digested with *Eco*RI; lane 6, pRSFO885 digested with *Bam*HI; lane 7, pJ1–8Str^{R}38 digested with *Eco*RI; lane 8, pJ1–8Str^{R}38 digested with *Bam*HI; lane 9, pS322 digested with *Eco*RI; lane 10, pS322 digested with *Bam*HI; lane 11, pS43 digested with *Eco*RI; lane 12, pS43 digested with *Bam*HI; lane 13, *Sau*IIIA digest of T7 DNA (length standards, gift from F.W. Studier). The 2.45 kb band in lanes 2, 10 and 12 contains two fragments of equal size.

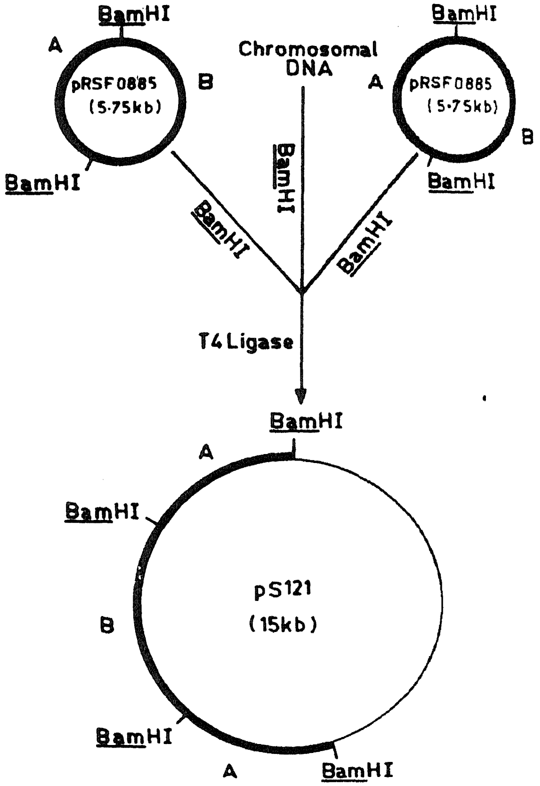

Figure 2. Schematic representation of the structure of pS121 and the orientation in which two copies of the *Bam*HI fragment A (2.45 kb) and one copy of fragment B (3.3 kb) of plasmid pRSFO885 are ligated to allow the insertion of a chromosomal DNA fragment.

Table 2 *Stability of plasmids derived from pRSFO885 and pJ1–8*

Plasmid	Per cent Amp^R stable
pS43	60.0 (145)*
pS121	72.0 (189)
pS322	93.3 (163)
pS436	86.1 (130)
pJ1–8	6.2 (193)
pRSFO885	96.4 (110)
pJ1–8Str^{R}38	3.8 (183)
pJ1–8Str^{R}14	19.0 (189)
pJ1–8Nal^{R}33	35.0 (177)

*Figures in parentheses are numbers of clones checked.

these are constructs from one or more combinations of the two *Bam*HI fragments containing a chromosomal insert with uptake sequences[14,15], which is lost following entry. Since the ligated DNA did give transformation initially these transformants may have lost the chromosomal insert present in the plasmids after plasmid entry into the cells. This led to the question of why plasmids pS43, pS121, pS322 and pS436 gave transformation for the *amp*^{r} marker. In the process of determining the molecular size of these four plasmids by the method of McDonell *et al.*[16], it was found that these four plasmids carried the small *Bam*HI fragment (2.45 kb) of pRSFO885 in duplicate (as indicated by the relative intensity of the band on agarose gels). Figure 1 shows the agarose gel electrophoresis of *Eco*RI and *Bam*HI digests of 3 of the 4 plasmids. Also shown are restriction digests of pRSFO885 and other plasmids. It was found that the molecular weights estimated from the *Bam*HI fragments were lower than the estimates obtained by using the *Eco*RI fragments. This discrepancy could be resolved if the small *Bam*HI fragment of pRSFO885 was considered to be present in duplicate (data not shown). Duplication of this fragment is required probably because both the *Bam*HI sites in pRSFO885 are in vital segments of plasmid DNA and the only way to hold an insert stably at a *Bam*HI site would be by having a fragment duplicated such that both sites are left intact and a third new site is available for insertion of foreign DNA. A schematic representation of this is shown in figure 2. It should be noted that the three *Bam*HI fragments have to be in the correct orientation for an extra *Bam*HI site to be available for insertion of foreign DNA in the presence of two functional *Bam*HI sites.

The four plasmids, and a few others, were tested for their stability. As shown in table 2, the four chimeric plasmids derived from *Bam*HI-cut pRSFO885 are more stable than the plasmids derived from pJ1–8 (with the exception of pKuvr1)[17]. The fact that all four plasmids carried only the small fragment in duplicate may be purely a coincidence.

A new principle of cloning DNA emerges from this i.e. even when the cutting site (of *Bam*HI) in the DNA may be in a gene with a vital function, the problem can be overcome by duplication of the intervening plasmid segments in the construct.

The help of Dr V. P. Joshi on several occasions is gratefully acknowledged.

16 January 1989

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TRANSFER OF SALINE TOLERANCE FROM ONE STRAIN OF RICE TO ANOTHER BY INJECTION OF DNA

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SALINITY and drought are regarded as the major problems facing rice production in the world. The moderately saline-tolerant variety of rice, Pokkali, is widely cultivated in the brackish water areas of Kerala, India and the sensitive variety of rice IR-20, is cultivated in the irrigated areas of the country. The tall and long-duration rice variety Pokkali cannot be adopted for cultivation under upland conditions.

Direct injection of genomic DNA of Pokkali into developing floral tillers of variety IR-20 produced transgenic seeds that were similar to Pokkali in husk colour, germinated well in 0.2 M NaCl, and had a 4–6-fold higher proline content. Transgenic seeds expressing chimeric genes have practical use in plant breeding to improve the quality and yield of cereal crops.

Genomic DNA (75 μg) isolated from rice variety Pokkali[1] and purified by gel filtration through a

Transgenic seeds
Pokkali' DNA

Biogel A5m column[2], in 1 ml of 10 mM Tris–HCl, 1 mM EDTA, pH 8.5, was injected into each of 4 floral tillers of rice variety IR-20 about 75 days after transplantation at the zero auricular stage when the inflorescence is about 2 cm. After about 45 days, mature seeds from injected and uninjected tillers were collected, dehusked, and spread on germination papers soaked in water or 0.2 M NaCl at 30°C for 36 h in the dark. About 13% of Pokkali and 17 out of 623 seeds from the injected tillers of IR-20, germinated. None of the 603 IR-20 seeds from uninjected tillers germinated in 0.2 M NaCl. Individual seeds were homogenized in 0.5 ml of 3% (v/v) sulphosalicylic acid, and an equal volume of a mixture of glacial acetic acid and 6 M orthophosphoric acid in the ratio 3:2 (v/v) containing 2.5% (w/v) ninhydrin was added. The mixture was heated on a boiling water bath for 1 h and cooled on ice. The proline–ninhydrin complex was extracted with 1 ml of toluene, and proline was estimated from absorbance at 520 nm[3].

The amino acid proline is known to act as osmoprotectant in cellular adaptation of plants to osmotic stress caused by drought or salinity[4–6]. Tolerance to salinity was tested by the ability of rice seeds to germinate and accumulate proline when kept in high concentration of NaCl solution. Over 66, 30 and 13% of the seeds of the rice variety Pokkali germinated in 0.1, 0.15 and 0.2 M NaCl respectively at 30°C in the dark. Only 5% of the IR-20 seeds germinated in 0.1 M NaCl, above which there was no germination. However, the vigour of germination as shown by the weight and shoot and root length of Pokkali seeds germinated in 0.2 M NaCl was lower than that of those germinated in the absence of salt, showing the stress caused by salinity on germination. The amount of proline was more than three-fold higher in Pokkali seeds germinated in 0.2 M NaCl than in seeds germinated in the absence of salt, while the variety IR-20 did not show such an increase (table 1).

Table 1 *Proline content of seeds of rice varieties IR-20 and Pokkali and of transgenic seeds*

	Proline (nmoles)	
Variety	0 M NaCl	0.2 M NaCl
IR-20	13.9 ± 0.6	14.3 ± 1.5
Pokkali	28.4 ± 3.6	97.1 ± 8.5
Transgenic	—	90.6 ± 7.5

Each value is the average for 6 seeds.

Direct delivery of DNA into living plant tissues has been developed to circumvent the problems of generation of plants from transformed protoplasts and the host-range restrictions of *Agrobacterium tumefaciens*. Use of high-velocity microprojectiles for delivery of DNA into *Allium cepa* epidermal cells was tried successfully[7]. DNA from Sea island cotton injected into axial placenta of glandless upland cotton about a day after pollination seems to have been expressed and inherited[8]. A plasmid containing aminoglycoside phosphotransferase gene under the control of nopaline synthase promoter was injected into developing floral tillers of rye plants; a few transgenic kanamycin-resistant seedlings which expressed the enzyme activity were obtained[9]. We obtained 927 mature seeds from IR-20 rice tillers that received Pokkali DNA by injection. These seeds were smaller than those of normal IR-20, and the colour of the husk of about 50% of the seeds was similar to that of seeds of Pokkali (figure 1). The aleurone layer of the seeds from injected IR-20 was darker than that of normal IR-20 seeds, but not as dark as that of Pokkali seeds. Of 623 such seeds, 17 germinated well in 0.2 M NaCl. The vigour of germination was comparable to that of Pokkali seeds germinated in 0.2 M NaCl. None of the 603 seeds from IR-20 tillers that did not receive Pokkali DNA germinated in 0.2 M NaCl. The amount of proline in six germinated (0.2 M NaCl) seeds from injected IR-20 was 124, 53, 120, 95, 60 and 89 nmoles,

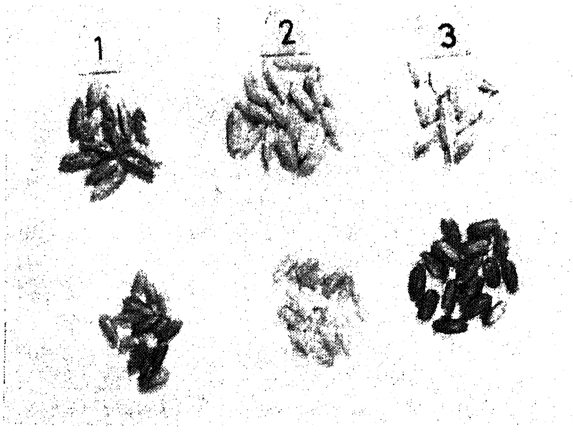

Figure 1. Seeds of (1) IR-20 plants that received Pokkali DNA, (2) normal IR-20, (3) Pokkali. Top row, with husk; bottom row, dehusked.

which are 4–8-fold higher than that of normal IR-20 seeds (table 1).

In various organisms, like bacteria, algae, crustaceans and higher plants, accumulation of proline in cells during water stress was found to prevent cell dehydration. The gene for γ-glutamyl kinase, which is the key enzyme in the biosynthesis of proline, may become insensitive to feedback inhibition by proline, resulting in overproduction of proline in Pokkali and transgenic rice seeds. Transfer of DNA from the same or a different species of plant by direct injection into floral tillers may find wide application in the generation of transgenic plants with specific qualities.

This work was supported by the Department of Biotechnology, Government of India.

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BOOK REVIEWS

Topics in Expert System Design: Methodogies and Tools, (ed.) G. Guida and C. Tasso (Published by North-Holland Elsevier Science Publishers BV, Amsterdam, the Netherlands), 1989, pp. 442, Price: US$97.25/Dfl. 185.00.

This book has tried to bring out the potential applications of expert system technology and artificial intelligence in decision making, planning, design, control, supervision and diagnosis. The editors have tried to address the fact that the lack of a sound reliable design methodology is responsible for the limited industrial impact of expert system designs. The book examines important emerging topics, advances in methodology and applications to specific practical problems. It consists of the following contributions: life cycle; domain evaluation; design techniques; development tools; knowledge acquisition and modelling; and validation and evaluation.

The book has covered in a lucid manner the major design aspects of expert system design and I am sure that it will make vital reading material for professionals. The book offers concrete guidelines to designers of expert systems and promotes basic and applied research on methodologies and tools. The collection also includes results of research carried out in the USA and Europe in expert system design.

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Laboratory Techniques in Cytogenetics and Plant Breeding, by S. S. Choudhary and Prabha Choudhary, (Published by Kalyani Publishers, 1/1 Rajender Nagar, Ludhiana 141 008, India), 1989, pp. 58, Price: Rs. 35.

This is a concise book on the practical aspects of experimental work in the field of cytogenetics and plant breeding. It meets the requirements of undergraduate and graduate students interested in pursuing cytogenetics in their career. The book serves especially as a ready reference for making cytological preparations. However, plant breeding, including mutation breeding, is dealt with very briefly. In the chapter on biostatistics, F test should also have been included since it is commonly used in yield evaluation experiments. The photographs of cytological preparations are of very poor quality and there is need for improvement. There are too many errors of spelling etc. which should be corrected in future editions.

I hope the authors' aim to encourage and stimulate experimental work in classical cytogenetics by the younger generation will be realized by this book. Present-day students aspire to work in the newly emerging areas of molecular biology and biotechnology. However, we will always require people willing to work in the classical fields of cytogenetics and plant breeding in order to continue our efforts to make further genetic improvements of crop plants and farm animals. From this viewpoint, the present book provides basic techniques required by students and researchers in the field of cytogenetics and plant breeding.

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Zoosporic Fungi of India, by R. Dayal and Usha Kiran, (Published by Inter-India Publications, D-17, Raja Garden Extension, New Delhi 110 015), 1988, pp. 297, Price: Rs. 250.

Zoosporic fungi have not been popular objects of research, particularly with Indian mycologists. A book on zoosporic fungi highlighting the research that has been carried out in India has therefore been long overdue. The authors should be praised for attempting this. The zoosporic fungi have been dealt with in two sections, on uniflagellate and biflagellate fungi. In each section the important morphological characters have been outlined. The detailed descriptions would have been more useful if complemented by figures. The methodology part has been dealt with extensively and reflects the experience of the authors. The chapters on the work done in India, however, have turned out to be disappointing, merely presenting summaries of papers in chronological order. An analysis of the directions this field of research has followed in this country, along with current trends and prospects, could have been attempted. Editorial mistakes are rampant. Reference to nineteenth century literature is made while citing twentieth century references (p. 22). 'Present evidence' is claimed to state an ascending order from Phycomycetes to Ascomycetes and Basidiomycetes (p. 108), while the former is now actually well accepted as being phylogenetically heterogeneous. Elsewhere fungi are referred to as a separate kingdom of the Plant 'kingdom'. I would have expected this book to be of ready value in identifying Indian zoosporic fungi. Although lists of Indian zoosporic fungi and keys to families are presented, one will still have to refer to the original publications and to monographs such as that on Indian chytrids by Karling for identification. On the whole, the book could have been edited to make reading less laborious. In spite of these drawbacks the book is a useful one, for it brings together the literature on this subject in India and draws the attention of mycologists to this group of interesting fungi.

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Thrips and Gall Dynamics, by T. N. Ananthakrishnan and A. Raman, (Published by Oxford & IBH Publishing Co. Pvt. Ltd, 66 Janpath, New Delhi 110 001), 1989, pp. 120, Price not mentioned.

This book is a timely addition to the literature on thrips, written by two well-known personalities, one a renowned entomologist and the other a botanist. Dr Ananthakrishnan has made so many contributions to the taxonomy and bioecology of Indian thrips that he should be rightly called the father of thrips research in India.

The book gives a vivid account of the economically important thrips species and their impact on host plants. The bioecological account of thrips species is extremely well documented. The information in this book will be of great help in the management of economically important thrips species. The account of the life cycle of thrips in total synchrony with the formation of galls has been beautifully presented from the viewpoint of their co-evolution. Good photographs and illustrations, with clear explanations, add to the quality and usefulness of this book. There is also a long table showing thrips species, their hosts and world-wide distribution. This book should prove very useful to zoologists, entomologists and botanists.

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THE SCIENTIFIC COMMUNITY

Awards and honours

Prof. Govind Swarup, Tata Institute of Fundamental Research, Bombay, is to receive the physics prize of the Third World Academy of Sciences, Trieste, Italy, for 1988. Prof. Swarup will receive the award (a cash prize and a medal) in Bogota, Colombia, on 16 October. The award is for Prof. Swarup's contributions to radio astronomy in the last thirty years. Prof. Swarup designed the Ooty radio telescope in Udhagamandalam (Ooty) and is now Director of TIFR's giant metre-wave radio telescope (GMRT) project, which hopes to complete construction of the world's largest radio telescope near Pune in 1992.

Appointment

Dr Sushil Kumar, Professor of Molecular Biology and Biotechnology, IARI, New Delhi, has been appointed Scientist G and Head, Human Resource Development Group, CSIR Headquarters, New Delhi (11 April). Dr Sushil Kumar worked on the organization of the tryptophan operon in *Salmonella typhimurium* and on phage lambda in the USA. At IARI he investigated the function of cAMP in *Escherichia coli*. His most recent work is in the genetical analysis of the symbiosis between *Rhizobium* and leguminous plants.

Dr R. A. Mashelkar has been appointed Director, National Chemical Laboratory, Pune (31 May). Dr Mashelkar's work is in polymer science and engineering, molecular and convective diffusion, non-Newtonian fluid mechanics and rheology.

Retirement

Dr L. K. Doraiswamy retired as Director, National Chemical Laboratory, Pune (31 May). Dr Doraiswamy has made significant contributions in chemical engineering thermodynamics and process development. Among his achievements are a continuous catalytic process for making dimethylaniline from aniline and methanol, a fluid-bed reactor for making chloromethanes from chlorine and methanes, a fluidized-bed method for making chlorosilanes from ferrosilicon and methyl chloride, and basic engineering designs for the commercial plant at Indian Petrochemicals Ltd (IPCL), Baroda, for making acrylates.

William Shockley passes away

William Bradford Shockley, co-inventor of the transistor with John Bardeen and Walter Brattain, died in Stanford, California, USA, on 12 August. Shockley was born in London on 13 February 1910 and had his higher education in the US. After obtaining a PhD in physics from Harvard University in 1936, Shockley worked at the Bell Telephone Laboratories (now AT and T Bell Labs) until 1955. He then headed the Shockley Semiconductor Laboratory at Beckman Instruments. In 1963 Shockley was appointed the first Alexander M. Poniatoff Professor of Engineering Science at Stanford University. He was elected to the National Academy of Sciences (USA) in 1951.

In 1946, at Bell, Shockley began work with Bardeen and Brattain on developing a semiconductor device for electronic amplification. The point-contact transistor, the field effect theory and the junction transistor were soon developed, and led to the electronics revolution. The invention of the transistor is a milestone in the development of solid state physics. Shockley also did research on energy bands of solids, ferromagnetic domains, plastic properties of metals, theory of grain boundaries, and order and disorder in alloys. For the invention and development of the transistor, he shared the Nobel Prize for physics in 1956 with Bardeen and Brattain.

In later years Shockley was also interested in the inheritance of intelligence and eugenics, but some of his ideas led to controversy.

EDITORIAL

RESPONSES TO THE ARTICLE ON SAC–PM

The article entitled 'Science Advisory Council to the Prime Minister—a brief overview' by Dr P. J. Lavakare (Current Science, **58,** *777) has evoked many responses from our readers, justifying our optimism that scientific issues would provoke debate among scientists.*

Many congratulated Current Science for publishing the article. We cannot take credit for this and have only to thank Dr Lavakare and the Chairman of SAC–PM for deciding to inform the scientific community through Current Science. To reproduce all the written and oral comments we received would be difficult and may even be repetitive. Instead, as samples we publish two letters, one, which made us sit back and think, from a teacher of English in a school, and another from a scientist (see p. 1000). We summarize below most of the comments we received.

i) Dr Lavakare deserves much praise for writing a fair and factual article soberly describing SAC–PM and its activities without any attempt to paint them in glowing colours.
ii) SAC–PM must be complimented on the manner in which it has proceeded to tackle the problems of science in India.
iii) SAC–PM is also to be congratulated on the considerable amount of work it has done, the number of recommendations it has made and the technical reports it commissioned and received. These reports should, if possible, be made available to a larger scientific public through journals (like Current Science) and feedback obtained from 'bench-level' scientists.
iv) A term of reference of SAC–PM (as given in the DST annual report) seems to have been overlooked—that it has to look into the problems of the Science Departments. If this is true, why are the budgets and activities of Space, Atomic Energy and Defence treated as sacred cows and not discussed? Nor have some of the important problems of some of the Science Departments been taken up.
v) Similarly, why has not the effectiveness (or otherwise) of autonomous Councils like CSIR, ICAR, etc. and their laboratories been a subject of study?
vi) It is unfortunate that no action has been initiated and recommendations made to stop the impoverishment of universities and to put them on new tracks for rejuvenation. This lacuna [according to some] is due to the fact that none of the members is really from a university [clearly these correspondents do not consider the Indian Institute of Science as a university in the strictest sense of the term!].
vii) The members of SAC–PM are obviously very good scientists who will uphold the type of science they are familiar with. But can the type of science they do be considered to be synonymous with the science that may be necessary, at least immediately, to improve the lot of the majority of our population, which is mostly in rural India and below the poverty line?
viii) The members of SAC–PM, judged from their public statements, subscribe to paradigms that became obsolete three decades ago. The Council does not have any theoretician in science policy (say like Weinberg) or a person who can perceive the philosophical and economic implications of science to a country like ours, where the majority of the population are in villages and pursue agriculture.

If this editorial summary is quoted out of context, one may get the feeling that most of the responses have been critical of SAC–PM. This is not true. The majority of the comments are extremely complimentary to SAC–PM, to Dr Lavakare and to Current Science.

Finally Current Science is asked to be wary of slowly becoming a mouthpiece of government and its machinery. With the warning is the hope that the editor will be able to withstand all external pressures and keep the journal 'non-aligned'. We shall of course do all we can to keep it that way.

SCIENCE NEWS

Discovery of asteroid number 4130

We report the discovery of a new asteroid, number 4130, from India. The last asteroid discovery from India was by Pogson in 1885. In fact Norman Robert Pogson discovered five asteroids between 1861 and 1885. The sky survey project[1,2] with the 45 cm Schmidt telescope has now led to the discovery of this asteroid. In figure 1 the arrow indicates the position of the new asteroid as observed on our plate taken on 17 February 1988.

This object was given the temporary designation 1988DQ1, and based on the preliminary orbital elements by Nakano[3] an ephmerides for future positions was calculated. At its next opposition in May 1989 it was recovered on our plates and these positions were communicated to the Minor Planet Center.

The complete listing of all observations from Kavalur for this object can be found in the Minor Planet Circulars[4,5] published on behalf of Commission 20 of the International Astronomical Union by the Director of the Minor Planet Center at Cambridge, USA. The final orbital elements together with the residuals (observed minus calculated positions) have been calculated by Nakano[3,6].

The apparent magnitude of the asteroid at the 1988 opposition was about 14.3 magnitudes. The orbital period is 5.34 years. The orbital plane is inclined to the ecliptic at about 9.80 degrees.

We thank Prof. T. Gehrels of the Lunar and Planetary Laboratory, University of Arizona, for his extremely helpful advice at the start of our search project for asteroids and the tenth planet.

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K. KUPPUSWAMY, A. PARANJPYE,
V. MOORTHY, R. VASUNDHARA

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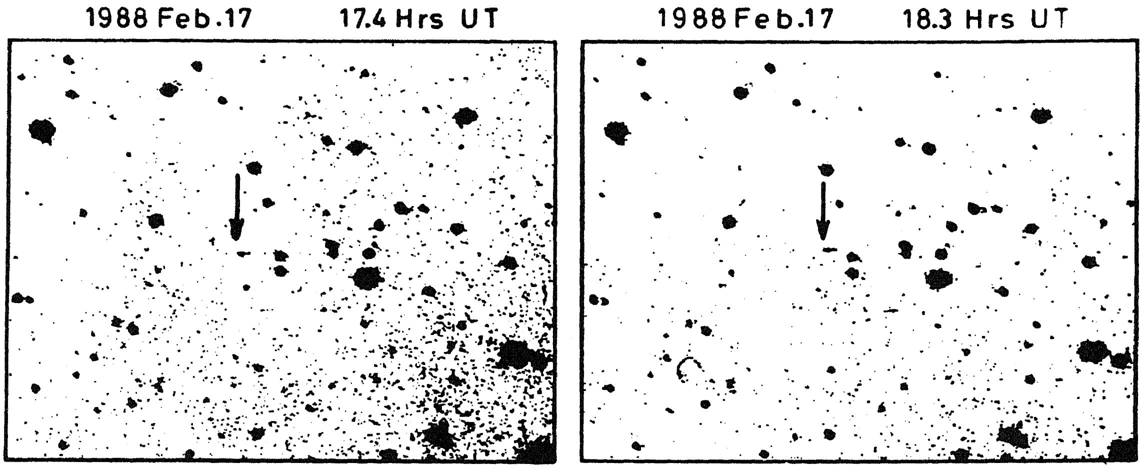

1988 Feb.17 17.4 Hrs UT

1988 Feb.17 18.3 Hrs UT

Figure 1. Asteroid Number 4130 discovered with the Kavalur 45 cm Schmidt telescope on 17 February 1988.

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6. Nakano, S., Minor Planet Circular Number 14777, 1989.

Cold fusion results from BARC*

Investigations of cold fusion phenomena carried out at Trombay during April–June 1989 have positively confirmed the occurrence of d–d fusion reactions in both electrolytic and gas-loaded Pd and Ti metal lattices at ambient temperatures. Neutron emission has been observed even when the current to the electrolytic cell is switched off or, in the case of gas-loaded Ti targets, when no externally induced perturbation, such as heating, cooling, evacuation, etc., is effected. The main findings of the Trombay investigations to date may be summarized as follows:

(i) Tritium is the primary product of cold fusion reactions, notwithstanding the fact that the tritium, if any, entrapped inside the palladium electrodes has yet to be quantitatively assessed. Thus cold fusion may be characterized as being essentially 'aneutronic' with a neutron-to-tritium channel branching ratio of less than 10^{-8}.

(ii) Neutron emission from both electrolytically loaded Pd and gas-loaded Ti is basically Poisson in nature, i.e. the neutrons are emitted one at a time. However, it is not clear whether the neutrons are generated in the d–d fusion reaction itself or are produced in a secondary reaction involving the energetic protons or tritons. In this context it would be of interest to look for the possible presence of 14 MeV neutrons in cold fusion experiments.

(iii) Occasionally nuclear events do appear to take place in which over a hundred neutrons are generated in a single sharp burst. Viewed in the light of the branching ratio estimate of 10^{-8} noted above, this leads to the intriguing conclusion that a chain reaction involving as many as 10^{10} fusion reactions occurs within a time span of 100 μs.

(iv) Autoradiography of gas-loaded Ti targets demonstrates in a simple and elegant manner not only the occurrence of cold fusion, but also the production of tritium. The estimated tritium-to-deuterium isotopic ratio in these targets is several orders of magnitude higher than in the initial stock D_2O and as such cannot be explained away on the basis of preferential absorption of tritium by the titanium, as may be suspected. The existence of highly localized regions (hot spots) on the target surface where tritium is concentrated, as well as the occurrence of spots all along the periphery of the Ti disc, point to the important role of lattice defect sites in the absorption process, or in the accumulation of tritium following migration after its formation, at least in titanium.

The very high probability for the tritium branch in cold d–d fusion reactions would indicate processes of neutron transfer across the potential barrier as postulated by Oppenheimer[1] over half a century ago and elaborated on more recently by Rand McNally[2]. If neutron transfer as envisaged by these authors does take place so easily, it may have many implications for the future of nuclear technology, for the deuterium nuclide might very well do the work that free neutrons do in present-day fission reactors. In the context of the emerging energy production scenario, aneutronic fusion reactions such as this may give rise to new fusion technologies, providing a cleaner energy source for the twenty-first century.

1. Oppenheimer, J. R. and Philips, M., *Phys. Rev.*, 1935, **48**, 500.
2. Rand McNally, Jr, J., *Bulletin of APS*, Baltimore Meeting, April 1983.

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*Summary of paper presented at the Fifth International Conference on Emerging Nuclear Energy Systems (ICENES V), Karlsruhe, FRG, 3–6 July 1989.

CORRESPONDENCE

SAC–PM

When I read in the newspaper that a write-up on the Science Advisory Council had appeared[1] in your esteemed journal, I borrowed a copy and read the article. I must congratulate you for publishing this article, written by no less a person than the Secretary of the Council. It was informative and written in a simple style, so that even a layman like me could understand how SAC functions and what it attempts to do.

There is one omission in the article, i.e. the names of the members of SAC. I assumed that most scientists of India would know. I met many from all over India at Ottappalam where a large national science exhibition was held recently. Very few scientists there knew the names of the members or even what SAC was and what it does. So *Current Science*, I feel, has done a service in educating scientists also! Incidentally you may be happy to know that many scientists *had* heard of *Current Science*; although, alas, a good many confessed that they do not read it! After some 'research' I was able to get the names, the affiliations and the scientific disciplines of the SAC members.

I have a serious fear. Please allow me to express it through your columns (if you accept letters from non-scientists). The members of SAC are, I am told, distinguished scientists. There can be no doubt that they will uphold science—the type of science they do and are familiar with, which may bring prestige to the country. The question that is uppermost in the minds of many (like me) is: *Can such sophisticated science be equated to the science that may be immediately necessary to improve the lot of the majority of our population who are in rural India and who are much below the poverty line?*

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[1] Lavakare, P. J., *Curr. Sci.*, 1989, **58**, 777.

K. K. Menon

Palghat

SAC–PM consists of:

Prof. C. N. R. Rao, Director, Indian Institute of Science, Bangalore—Chairman; Prof. V. L. Chopra, Head, Biotechnology Centre, Indian Agricultural Research Institute, New Delhi; Prof. Madhav Gadgil, Centre for Ecological Sciences, Indian Institute of Science, Bangalore; Dr A. S. Ganguly, Chairman, Hindustan Lever Ltd, Bombay; Dr R. A. Mashelkar, Director, National Chemical Laboratory, Pune; Prof. R. Narasimha, Director, National Aeronautical Laboratory, Bangalore; Prof. J. V. Narlikar, Director, Inter-University Centre for Astronomy and Astrophysics, Poona University, Pune; Mr Sam Pitroda, Adviser to PM on Technology Missions, New Delhi; Dr Sekhar Raha, Chief Executive, ICI India Ltd, Fertilizer Division, New Delhi; Dr P. Rama Rao, Director, Defence Metallurgical Research Laboratory, Hyderabad; Dr P. N. Tandon, Dept of Neurosurgery, All India Institute of Medical Sciences, New Delhi; Dr P. J. Lavakare, Adviser, Department of Science and Technology, New Delhi—Secretary.

—Editor

Please accept heartiest congratulations on behalf of all working scientists and those who keenly watch and are interested in the growth of Indian S&T for (a) the sensitivity expressed in your note prefacing the welcome article of Dr P. J. Lavakare, and (b) introducing the section 'News from Delhi' which, if taken in the right spirit, will hopefully provide a creative/constructive forum for feedback, dialogue and exchange of ideas and issues between policy makers, policy handlers and 'grass-roots' workers.

This may indeed fill a long-felt gap in communication on matters of S&T. Following such an approach, I am enclosing a response to Dr Lavakare's article in the hope that you may find it of sufficient interest and significance for readers of your esteemed journal.

U. Raval

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The points raised by Dr Raval have been included in the editorial in this issue.

—Editor

Science and audit

I was very happy to read the reactions[1,2] on science audit. Such a dialogue is necessary to evolve appropriate rules, procedures and systems. I hope the healthy discussion can be continued through your columns. May I now clarify some issues that have been thrown up.

The major issue is about delays in achieving the goals. Audit does not comment upon marginal delays or where there are sufficient reasons. But Audit would be failing in its duty if it did not tell the people about substantial delays and cases where science management has been found wanting. Time is of the essence in science and it is one of the parameters for any project or programme. If science managers have not taken all the constraints of the environment into account and an optimistic schedule has been drawn up, it is unscientific and unrealistic, and creates idle assets and causes postponement of the flow of benefits to the people. Sometimes such schedules also result in diversion of resources to a less attractive project among alternatives. Any nuclear power project is a case in point.

As regards institutional delays as distinct from individual or isolated delays, my refrain would be that scientists should devise new sets of rules and procedures that would be specific to their requirements. Thus it is not necessary for directors of various national laboratories to chase the same supplier for repair or speedy supply of similar equipment. A centralized buying procedure or blacklisting of bad suppliers should save a lot of time and cost. Responsibility for evolving these rules and procedures lies with the scientific community. In other words, just as modern astronomy cannot be done with ancient telescopes, science cannot be carried on with outdated, 'colonial' rules and procedures.

Now, as regards availability of money, my observations were limited to government scientists and departments. One can see from the Audit Reports on National Laboratories that a mere 5% test-check has revealed any number of unoccupied staff quarters and scientists' apartments in various parts of the country. Similarly, equipment worth crores of rupees lies unpacked for years, or unused after the sponsored research is over. Also, when new projects are drawn up, say under international assistance, new sets of equipment, which are already available, are sometimes again listed for purchase. When financial grants are made on a liberalized scale, there is excessive expenditure on non-scientific items. I am not against incentives for scientists but idle investments must be avoided.

Regarding excessive control and loss of autonomy, let me assure the scientists that this is because the collegium mode and cluster model have been replaced with empires of science. In sum, was it not the view of Sir C. V. Raman, who belonged to a financial service as well as to the scientific community, that some national laboratories are mausoleums of Indian science? This must be avoided. In addition to science missions, we need missions in science to underpin the era of expansion.

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1. Venkataraman, G., *Curr. Sci.*, 1989, **58**, 673.
2. Pappu, S. V., *Curr. Sci.*, 1989, **58**, 782.

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The following quotations may be interest to readers:

'Personally, speaking for myself, I am of the opinion that this dignitary or officer [CAG] is probably the most important officer in the Constitution of India. He is the one man who is going to see that the expenses voted by Parliament are not exceeded, or varied from what has been laid down by Parliament in what is called the Appropriation Act. If this functionary is to carry out the duties—and his duties, I submit, are far more important than the duties even of the judiciary—he should have been certainly as independent as the judiciary. But, comparing the Articles about the Supreme Court and the Articles relating to the Auditor-General, I cannot help saying that we have not given him the same independence which we have given to the judiciary, although I personally feel that he ought to have far greater independence than the judiciary itself'.

[B. R. Ambedkar]

'Ours is a poor country, its resources are limited and we cannot afford to risk any kind of waste, and, therefore, the Audit Department will have to look upon its functions as functions of the greatest public utility by pointing out errors and by showing where and how we can remove abuses, effect economy, increase efficiency and reduce waste of expenditure'.

[S. Radhakrishnan]

—Editor

ANNOUNCEMENT

FINANCIAL SUPPORT FROM INSA FOR PARTICIPATION IN CONFERENCES

The Indian National Science Academy invites applications from Scientists for partial financial assistance to participate in important Scientific International Conferences abroad during the year 1990–91. Scientists interested to avail of financial support from the Academy for participation in International Conferences to be held during the year 1990–91 are requested to apply in the prescribed proforma. The proforma is available from the Office of **The Executive Secretary, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002.**

The scheme is operated under three different categories, viz. (1) For International Conferences sponsored by the International Council of Scientific Unions (ICSU) and its affiliated bodies (ICSU Conferences); (2) For International Conferences sponsored by other agencies (Non-ICSU Conferences); (3) For applicants who are interested in partial travel assistance only, for participation in any International Conferences abroad. The applicants must be below 35 years of age. This is categorized as INSA–COSTED Travel Grant.

Category 1: Scientist who has been invited to deliver a plenary lecture/preside over a session of a scientific conference or whose paper has been accepted for presentation, and who will also be provided maintenance allowance during his/her stay abroad and partial travel expenses by some agency, will be given preference over others. INSA's financial support, in case of selection is limited to a maximum of 50% International travel, 50% maintenance allowance for the duration of the conference and registration fee, wherever necessary. The prescribed application form duly completed should be despatched **latest by January each year** for consideration by a Committee of the Academy during the year.

Category 2: Scientist who has been invited to deliver a plenary lecture/preside over a session or whose paper has been accepted for presentation and who will also be provided maintenance allowance during his/her stay abroad and partial travel expenses by some agency, will be given preference over others. INSA's financial support in case of selection is limited to a maximum of Rs 7,500/- only. Applications for this category should be received in the Academy for consideration **three months prior to the date of commencement of the Conference.**

Category 3: Under this category the scientist who is interested to avail partial travel assistance only, for participation in any International Conference and is also below 35 years of age may send his/her formal request on a prescribed application form **at least three months prior to the date of commencement of the Conference.** The applicant should fulfill the following eligibility criteria before sending his/her application:

(a) His/her age should be below 35 years on the date of commencement of the Conference.

(b) He/she should hold a Doctorate degree.

(c) His/her paper should have been accepted for presentation.

(d) His/her local hospitality and partial travel expenses has to be borne by the organisers or some other agency.

Candidates selected will be supported partially for their travel cost, jointly by INSA and COSTED (Committee on Science & Technology in Developing Countries). The applications will be screened once a quarter during the year.

MEETING REPORTS

XII AIRAPT HIGH PRESSURE CONFERENCE

The XII International Conference on High Pressure Science and Technology was held at the University of Paderborn, FRG, from 17 to 21 July 1989 under the auspices of AIRAPT (a French acronym for the International Association for the Advancement of High Pressure Science and Technology). Over 375 papers, about 200 in poster sessions, were presented. The topics covered were high pressure studies in the area of biology, chemistry, solid state physics, polymers, liquid crystals, fluids, and geophysics and planetary physics. Various aspects of high pressure engineering and safety, methodology and techniques for static and dynamic high pressures, and synthesis of superhard materials were also covered. The abstract booklet contains 15 papers from India.

Biological studies

A number of papers presented dealt with pressure effects on proteins and biomembranes. Model phospholipid biomembranes have been studied under moderately high pressure–high temperature conditions, using high-resolution NMR, infrared spectroscopy and neutron diffraction. According to a number of papers presented at this conference, high pressure studies on these materials have thrown considerable light on various biomedical aspects, such as mechanisms of anaesthesia and alcohol-induced liver cirrhosis, structural and dynamic properties of cancer cells, pressure–temperature phase diagram of phospholipids, etc. Raman spectroscopy of protein samples under pressure has yielded interesting results on conformational changes and denaturation of proteins. Studies of pressure effects on surface-enhanced resonance Raman scattering promises greatly improved sensitivity in the study of these materials under pressure.

Chemical studies

Studies of pressure effects on chemical reaction kinetics were reported to have led to a better understanding and systematic classification of solvent exchange and ligand substitution reactions of octahedral complexes of transition metals. Some of the other reactions studied under high pressure were Diels–Alder reaction, (C–H–N) hydrogen transfer reaction and ring closure reactions of transient $W(CO)_5$ chelate complexes. Application of pressure was reported to have facilitated the synthesis of valuable heterocyclic organic compounds such as diazocoronands and cryptands. Interesting applications of high pressure in organophosphorus and macrocyclic chemistry were reported.

High T_c superconductors

A number of papers dealt with the pressure dependence of transport properties, lattice parameters and T_c of various high T_c superconductors. A study of P–T–x phase diagram of Y–Ba–Cu–O system under high oxygen pressures of up to 0.3 GPa was reported. Two new bulk superconducting phases, $YBa_2Cu_{3.5}O_{7+x}$ and $YbBa_2Cu_4O_{8+x}$, were reported.

Metallic (?) hydrogen

Results of optical measurements on hydrogen under high pressures of up to 250 GPa (2.5 Mbar) at 77 K were reported. The absorption and Raman spectra provided evidence that the electronic excitations in the visible region began at ~200 GPa. At 250 GPa hydrogen samples were opaque. The optical data were consistent with a band-overlap mechanism of metallization.

Metastable phases

The high-pressure phases of binary alloys such as Zn–Sb, Cd–Sb, In–Sb, Ga–Sb and Al–Ge can be retained metastably at liquid nitrogen temperature for extended periods of time. On heating at 1 atmosphere, the samples attain equilibrium state via different intermediate states, including amorphous state.

Quasicrystals

Formation of quasicrystalline Al_4Mn and Al_6Cr upon quenching the melts under high pressure was reported. Two different phases of Al_4Mn were obtained by changing the pressure. Quenching of melt at high pressure was reported to yield a new phase of Mn_4Si which exhibited twelve-fold symmetry.

Phase transitions

Pressure-induced phase transitions in a variety of compounds and elemental solids were reported. The semiconductor compound GeTe was examined under pressures of up to 25 GPa, and was found to undergo a continuous rhombohedral–cubic phase transition. The lattice parameters c and a of the hexagonal cell (associated with the rhombohedral cell) decrease continuously in the entire pressure range, but the c/a ratio registers a discontinuity at the transition. New phases of silica were reported under high-pressure and high-temperature conditions. On heating α-quartz or fused silica at pressures above 100 GPa a phase with $CaCl_2$-type structure was obtained. On heating cristobalite under pressure new phases appeared at 10 GPa and 30 GPa.

Pressure in 20,000 GPa range

Generation of dynamic pressure in the 20,000 GPa range in an underground nuclear explosion and the measurement of equation of state (EOS) of aluminium, iron and lead were reported. The inadequacy of the Thomas–Fermi model in predicting EOS at such high pressures and the necessity of using modern quantum-mechanical models based on the shell structure of atoms were pointed out. EOS data for a mixture of water, ammonia and isopropanol, and data on electrical conductivity of hydrogen in the pressure range 100–200 GPa were presented. These data provide important inputs in modelling the planet Uranus.

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SECOND INTERNATIONAL CONFERENCE ON ANALYTICAL CHEMISTRY IN NUCLEAR TECHNOLOGY

The meeting was held in Karlsruhe, FRG, from 5 to 9 June 1989. It focused on analytical techniques applied to the nuclear cycle. Many new laser-based techniques, such as time-resolved spectrofluorimetry and photoacoustic spectroscopy, were discussed. There were also papers on non-destructive and in-line analytical techniques such as multiplexed fibre-optic spectrophotometry, in-line flow coulometry and X-ray absorption edge densitometry.

Two papers from the Indira Gandhi Centre for Atomic Research, Kalpakkam, were also presented (T. R. Mahalingam). These were on the determination of trace metals in uranium by ICP–MS, and on the application of an electrochemical hydrogen meter in studies of reactions in liquid sodium.

OPINION

PROBLEMS OF SCIENTIFIC PUBLICATIONS

We publish below an article that appeared in The Guardian Weekly (August 6, 1989), which is the English translation of an article in Le Monde. It is a report (by Jean-Paul Dufour and Franck Nouchi) of an interview with Hubert Curien, the distinguished scientist, crystallographer and administrator who was in charge of many science departments, including the Department of Space, in France, and is now Minister of Research and Technology. He raises many problems that French science faces—the vast power exercised by a few 'powerful' journals that dominate the scientific publication market; the problem of refereeing papers; the problem of scientists and the news media; the involvement of Government in establishing journals; and the process of assessing scientists and scientific laboratories. The article brings out the fact that India is not the only country that is greatly concerned with its scientific journals and the publication of scientific papers. (In the reproduction here, we have added italics for emphasis.)

—Editor

'If God tried to enter CNRS (National Scientific Research Council), he'd flunk' quipped Hubert Curien, himself a scientist and now Minister of Research and Technology. 'He performed an interesting experiment, but nobody has ever succeeded in replicating it. He has explained his work in a voluminous publication, but it was not even in English, and he has published nothing since.'

The witticism has the merit of clearly raising the question of evaluating and publishing scientific research. This question was brought up this year by two sensational controversies—one a claim by a Frenchman, Dr Jacques Benveniste, that water retains the molecular memory of substances even in infinitesimal dilutions, and the second, an assertion by two scientists, an American and a Briton, that they had succeeded in achieving cold nuclear fusion. These controversies illustrate the dominant part that some scientific journals play in disseminating the work of research scientists by confirming their discoveries or exposing their errors.

The researcher who believes he has made an interesting discovery in the secrecy of his laboratory must communicate it to fellow scientists the world over who will then try to reproduce the experiment. If the results are positive, they in turn will publish their conclusions and only then does the original researcher's 'proposition' become a discovery that may at some future date rate a Nobel award. There are two major snags in the procedure: first, the conservative character of one section of the scientific community, and secondly, *the vast power exercised by a few big journals which dominate the scientific publications market.*

It is possible as of now to draw a few conclusions from the Benveniste case. In an interview he gave *Le Monde*, Hubert Curien *raises a number of questions concerning the part played by big international scientific journals and makes an appeal to Europeans to establish their own high quality scientific publications so as to counterbalance the hegemony of the English-speaking world in this area.*

Q. What are the lessons you draw from the Benveniste case?

A. Basically, three. First, it seems to me necessary to think about a scientific journals policy. It is extremely important. Secondly, we need to consider more thoroughly the question of evaluating research. And lastly, *it would seem to be desirable to set up a system for regularizing relations between scientists and the media.*

Q. In a book entitled *Les Explorateurs de la sant* (*Health Explorers*), to be published in a few weeks, Philippe Lazar, of INSERM (National Health and Medical Research Institute), points to the discretionary powers that big scientific journals have in accepting or rejecting the conclusions of scientific laboratory research. He wonders whether this does not entail the danger of genuinely original work being brushed aside.

A. It's a tricky question. Like many of my fellow scientists, I have been a member of refereeing committees of several major scientific journals. So I have some practical experience in this sort of thing. Very often, three kinds of articles are encountered: bad articles, which can be dismissed without difficulty; good articles, which can easily be passed over too quickly; and a fairly large number of articles whose utility no one is quite sure of, articles about which someone can one day say, 'Yes, I've read the article, it's not even false.' In general there is no objection to publishing such articles for their authors have put in honest work. There then remain those surprise articles, like Benveniste's contribution, which don't fit into any category. Obviously they need to be approached differently.

In this connection I recall the article that Otto Hahn and Friedrich Strassmann published in 1939 in which fission was mentioned for the first time. The writers, both excellent physicists working in Berlin, ended their article with the following remark, which I paraphrase: 'We feel that what we publish here conforms with nothing that is acceptable to our fellow physicists. It is possible we are mistaken. If that is the case, dear colleagues, do not treat us as idiots. Bear with us.

Q. Are you suggesting that Dr Benveniste should have followed the same course?

A. What I'm saying is that when you publish surprising things, you must keep a very open mind and remind yourself that you may well be mistaken, and appeal to fellow scientists to confirm or invalidate your own findings.

But to get back to scientific journals, the danger is that if we don't look out, the number of such publications which have any authority in the scientific market will diminish, leaving only American journals in the field.

Like everybody else, I'm of course distressed to note that fewer and fewer scientific articles are published in the French language. It's a problem. Another is whether the French and, more generally, the Europeans, will retain control of any major international journal. In my view, this is an even more important matter. Having control of an international journal is a matter of the greatest importance because, *whatever one may say, an American publication will not put the stress on the same things or have the same thrust and mix of articles as a European counterpart. A major Europe-based international journal for each of the principal sectors of science appears to me to be a fundamental objective.*

Q. In this connection, do you think that Britain is part of Europe?

A. There are big British international journals, like *Nature*, for example. Let's stay with continental Europe. Your remark is quite appropriate. British scientists often feel closer to their American than to their European colleagues. It's not clannishness, but a matter of disposition and mindsets.

Q. Is it up to the government to facilitate setting up journals of this sort and decide in what language they should be published?

A. The government should of course facilitate the establishment of such journals. It pays researchers, buys the equipment and provides operating credits. It's therefore quite natural that it should help establish publications.

The question of language is a difficult one. It must be realized that the scientific output of the French-speaking amounts to roughly 8 per cent today. Which is good. But among the other 92 per cent of science producers, there isn't a single researcher who doesn't know English. You have to face the facts.

Some French researchers exhibit a ridiculous coyness in believing that if they are not read or heeded it's because they speak French. *It is probable that the reason they are not read is because they're not 100 per cent convincing.*

What should be banned is a European journal, subsidized by the French government, ruling that it will not accept articles written in French. But such a journal deciding, on the contrary, that it will publish nothing except in French is beyond me.

Q. Are you in a way appealing to research scientists to be more public-spirited?

A. Our researchers must be able to continue publishing as they please in major English-language scientific journals. That's clear. They must be allowed the greatest freedom to publish where they like. Yet publishing in an English-language journal must not be made a sovereign remedy. If Europeans show that they have sufficient strength to publish journals of the same high quality, then it becomes just as interesting to have articles appearing in such publications. *The difficulty with an international journal is not so much launching it as keeping it going.*

Q. Are you going to take concrete measures to this end?

A. We do support scientific publications. It's important to make a distinction among the various kinds of publications. In the first place, there are journals publishing raw data where scientists should very quickly be able to publish their findings in a form more or less usable by their colleagues but possibly unusable by readers not specializing in the area.

Next, there are journals which carry scientific backgrounders and syntheses for a very well-informed but not necessarily specialist readership.

Lastly, there are journals for the public at large. There are good quality magazines of this sort in France. On the other hand, we don't have enough French-language journals specializing in syntheses. We have launched a highly appreciated journal, *Médecine-Sciences*, in cooperation with the Canadians. Consequently, we would be very receptive and make the necessary effort if we are approached with proposals in areas other than medicine and biology.

Journals publishing primary findings ought to be multilingual. In any case most of the papers will be in English. We have no illusions on that score.

Q. Let's go back to the report, which you describe as surprising. Should special publication procedures be provided for them?

A. That's up to the publishers of the journals. Let me just say that John Maddox, editor of *Nature*, showed a rather insular Machiavellianism in his handling of the Benveniste case.

Q. How do you judge the evaluation procedures currently prevailing in France?

A. There are at least two kinds of evaluation: evaluation of individuals and collective evaluation. Is a given laboratory good on the whole? Has a given research body a good output? Is a given research funding mechanism properly adapted? Should our country become more involved in this or that particular research area?

If we go back to individuals, it's clear the best way to judge a work of research is on actual evidence, that is to say basically on what has been published, but keeping in mind that, given the way scientists live today, published work is not everything. There is also — and this is very important — the way researchers can present their work at congresses or at conferences to which they have been invited. The latter system is very widespread in the United States, and Europeans have several such projects in the pipeline, one of them currently being examined by the Fondation Européenne de la Science. I'll be happy to see it produce results.

I should like to sound a word of caution about utilizing published work as a yardstick, in particular against the fad (happily already on the way out), of quantifying the evaluation of researchers on the basis of the volume of published material. In this method, the number of articles and the number of references to such articles are totted up and coefficients allotted more or less all round. True, not everything is bad in this system, but misusing it is. It in fact opens up possibilities of making considerable mistakes. One must beware of the effect of fashions and the formation of clans ('I'll quote you, and you quote me'). *The practice of making evaluations on the basis of the mass of published material requires to be treated with a good dose of humour. The unfortunate thing is that the most well-known enthusiasts of the method frequently appear to have no sense of humour at all.*

I know of no really rational system of quantitative evaluation. One has to depend on a kind of consensus in the scientific community. But even here we need to examine things closely. Who should sit on committees to evaluate researchers? Full-time research workers? By definition, they have very limited time in view of the fact they are themselves busy working on their own projects. Assessors whose active research careers are behind them? They will judge with the greatest honesty, but may use observation and (older) scientific models such as they had constructed as frames of references.

We're often told that people sitting on evaluation committees are too old. Well, that's true, but often because the youngest who have been approached have refused to take part. Scientists can't be asked to spend too much of their time judging others. There isn't a perfect evaluation system. Which is why it's absolutely necessary for confidence to reign in the research community.

ARTICLES

THE ISOLATION AND EXPRESSION OF POLLEN-EXPRESSED GENES*

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INTRODUCTION

POLLEN is the male gametophyte or male gamete-bearing structure, while the plant itself is the sporophyte or vegetative generation in the life cycle of flowering plants. Pollen develops following meiosis, is haploid, and is relatively simple in morphology, consisting of just three cells at maturity, viz. the vegetative cell and two sperm cells. Figure 1 shows the various stages of pollen development in maize. The function of pollen is the production of two sperm cells and their conduction through the pollen tube into the vicinity of the embryo sac in the ovary, where double fertilization can occur.

All the information necessary for the formation of a morphologically normal plant is present in the haploid pollen nuclei since it is possible to culture immature pollen in the test tube to reverse its normal development and obtain haploid embryos and plants. These haploid plants are similar in their morphological development to normal diploid plants. In the regular formation of the male gametophyte, however, one obtains only a very reduced morphology consisting of a tube in which the main biochemical events are related to pollen tube cell wall synthesis and the synthesis of cell membrane to keep up with the increase in length of the tube. Since the pollen grain–pollen tube system is relatively simple it might be easier to understand, compared to the morphologically much more complex vegetative systems, such as roots, stems and leaves.

MACROMOLECULAR SYNTHESIS IN POLLEN DEVELOPMENT

The mature pollen grains of several plant species have been shown to contain a large store of presynthesized messenger RNAs (mRNAs) (table 1). These mRNAs have been isolated, and shown, in cell-free translation systems, to code for polypeptides that appear to be similar on the basis of size and electrophoretic mobility to the proteins made during pollen germination and tube growth[1,2]. Experiments using inhibitors of RNA and protein synthesis suggest that these mRNAs are utilized during germination and early pollen tube growth (see review in ref. 3).

Large amounts of ribosomal RNA are synthesized prior to microspore mitosis. Following microspore mitosis, a sharp decrease occurs in ribosomal RNA synthesis, and the ribosomal

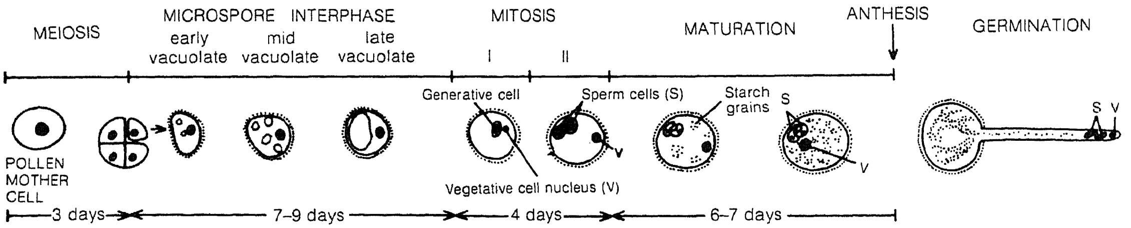

Figure 1. Diagrammatic representation of the various stages in the development of maize pollen. Mitosis I and II refer to microspore mitosis and generative cell division respectively.

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*Based on the text of the First Professor Nuggihalli Narayana Memorial Lecture delivered by the author on 23 August 1988 at the Indian Institute of Science, Bangalore.

Table 1 *RNA content of pollen grains of three plant species*

	(pg per pollen grain)	
	total RNA	poly(A)$^+$ RNA
Tradescantia paludosa[12]	196	5.1
Zea mays[2]	352–705	8.9–17.8
Nicotiana tabacum[13]	230	6.2

genes become transcriptionally inactive during the terminal stages of pollen maturation and subsequent pollen tube growth (see review in ref. 3). Transcription of the transfer RNA genes also follows a similar pattern[3]. Proteins, however, are synthesized during pollen germination and tube growth, utilizing the ribosomes and transfer RNAs made and accumulated during pollen maturation. Many pollens contain all the necessary proteins, mRNAs, ribosomes, etc. for rapid germination and initial tube growth on the stigma of the pistil. From the time the pollen grain is released from the anther to the time of its deposition on the stigma it is in an inhospitable environment. The trend in evolution has accordingly been towards pollen grains that contain all the macromolecules necessary for rapid germination, to enable the tube to penetrate the stigma and enter the style, where conditions are favourable for further development.

CLASSES OF POLLEN mRNA

Since the mature pollen grain contains mRNAs that have functions during germination, it was of interest to know how many different genes were transcribed to produce these mRNAs. Based on the kinetics of hybridization of [^{3}H]cDNA with poly(A)$^+$ RNA in excess (RNA C_0t analyses), estimates have been made of the numbers of different mRNAs present in the mature pollen grains of *Tradescantia paludosa* and of maize[4,5] (table 2). The mRNAs in mature *Tradescantia* pollen grains consist of three abundance classes with complexities of 5.2×10^4, 1.6×10^6 and 2.1×10^7 nucleotides. Approximately 15% of the mRNA is very abundant and consists of about 40 different mRNAs, each present on an average in 26,000 copies per pollen grain. The major fraction of the mRNA (60%) is made up of 1400 different sequences, each present in about 3400 copies per grain. The least abundant fraction is relatively small (24%) but is made up of 18,000 different mRNAs, each present on an average in 100 copies per pollen grain. The total complexity of *Tradescantia* pollen poly(A)$^+$ RNA is 2.3×10^7 nucleotides, which is equivalent to about 20,000 different kinds of mRNAs. In contrast, in shoots the complexity of mRNA is 3.4×10^7 nucleotides, which corresponds to about 30,000 different mRNAs. This indicates that approximately 60% as many genes are expressed in pollen as are expressed in vegetative shoots.

Very similar results are found with maize pollen; the mRNAs are the products of about 24,000 different genes[5]. The hybridization studies with maize and *Tradescantia* indicate that a large number of genes are transcribed in pollen. The 20,000–24,000 different mRNAs

Table 2 *Summary of homologus cDNA–poly(A)$^+$ RNA hybridizations from pollen of Tradescantia paludosa and maize*

	Fraction of total poly(A)$^+$ RNA		Complexity (nucleotides)		No. of diverse mRNAs		No. of copies per sequence per pollen grain	
Component	*T. paludosa*	Maize	*T. paludosa*	Maize	*T. paludosa*	Maize	*T. paludosa*	Maize
1	0.15	0.35	5.2×10^4	2.1×10^5	44	245	26,000	32,000
2	0.61	0.49	1.6×10^6	6.4×10^6	1,400	6,260	3,400	1,700
3	0.24	0.15	2.1×10^7	1.8×10^7	18,000	17,250	100	195
Total	1.00	1.00	2.3×10^7	2.5×10^7	19,444	23,755	—	—

Data taken from references 4 and 5.

found in mature pollen represent, as will be discussed later, mostly genes that are activated late in pollen development. It is likely that additional genes might be active immediately after meiosis and during the early stages of pollen development.

The mRNAs found in pollen are very abundant, i.e. are present in many copies. Even the least abundant mRNAs are present in 100–200 copies per pollen grain (table 2), whereas in shoots this fraction is present in 5–10 copies per cell[4,5]. This abundance of mRNAs in mature pollen would seem to suggest a requirement for the rapid synthesis of a large quantity of protein product during the terminal stages of pollen maturation, and/or germination and early pollen tube growth.

POLLEN-SPECIFIC GENES

Our results suggest that a large number of genes are expressed during pollen development. It is important to know how many of these genes are specifically expressed in pollen and not in other tissues of the plant. Heterologous hybridizations of pollen cDNA with shoot poly$(A)^+$ RNA and shoot cDNA with pollen poly$(A)^+$ RNA indicate that a minimum of about 65% of the pollen mRNAs are similar to mRNAs found in shoots. Because of various constraints in this sort of analysis, the estimate of pollen sequences shared with vegetative tissues could be[5] in excess of 90%. We have estimated, based on colony hybridizations of clones from cDNA libraries made to pollen mRNA with labelled cDNAs from pollen and vegetative tissues, that approximately 10% of the total sequences expressed in pollen are pollen-specific[6]. These estimates of the degree of overlap of the set of genes expressed in pollen with that of other tissues are in agreement with estimates made by others in the literature[7–9].

These results indicate that the genetic programme expressed during pollen development is extensive and that there is a substantial overlap between the set of genes active in the sporophyte and that in the male gametophyte.

In order to further our understanding of pollen development it was essential for us to isolate genes uniquely expressed in pollen and also genes expressed both in pollen and in vegetative tissues. We have accordingly constructed two cDNA libraries that represent the mRNAs expressed in pollen, one from *Tradescantia* and the other from maize, using standard recombinant DNA procedures. Each library consists of several thousand clones initially selected on the basis of their hybridization to ^{32}P-labelled cDNA made to poly$(A)^+$ RNA from pollen[6]. A few of the clones were pollen-specific; the majority were expressed in pollen and in vegetative tissues.

Figure 2 shows the pollen specificity of two clones, Zmc26 (*Zea mays* cDNA clone 26) and Tpc70 (*T. paludosa* cDNA clone 70), which hybridize in RNA blot hybridizations to mRNA from pollen and not to RNA from other tissues. Figure 3 shows another pollen-specific clone from maize (Zmc13). Representative 'shared' clones are shown in figure 4;

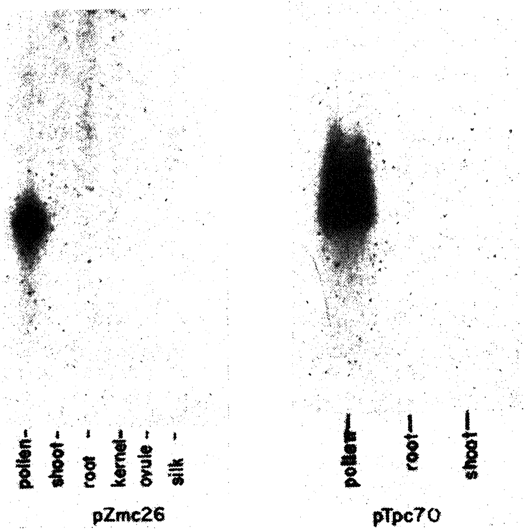

Figure 2. RNA blot (Northern) hybridizations to demonstrate specificity of the clones pZmc26 and pTpc70. Five μg of total RNA isolated from different tissues of maize and *T. paludosa* was hybridized with ^{32}P-labelled pZmc26 and pTpc70 respectively, followed by autoradiography. (Figures 2, 4, 5, 6, 7 and 8 are reprinted with permission from ref. 6.)

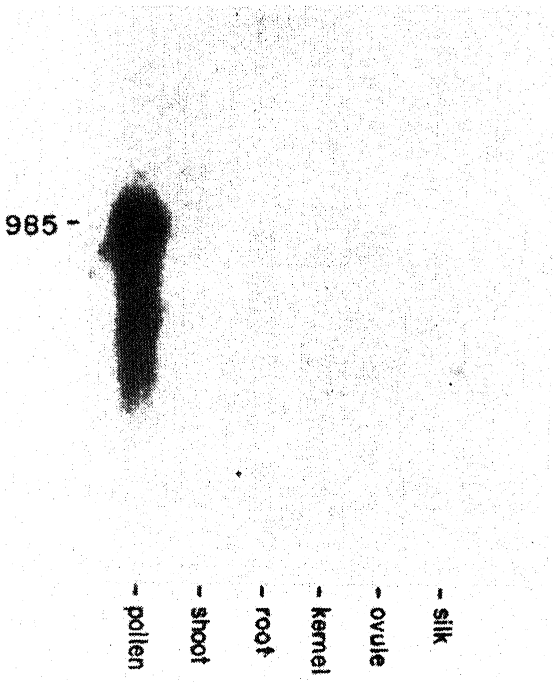

Figure 3. RNA blot hybridization to demonstrate pollen specificity of the clone Zmc13. Total RNA (5 μg per lane) isolated from the indicated tissues of maize was hybridized with ^{32}P-labelled Zmc13. The autoradiogram was overexposed to show clearly the lack of hybridization in the non-pollen lanes. (Reprinted with permission from ref. 10.)

these clones hybridize to RNA from pollen and also to RNA from one or more vegetative tissues.

Using these clones it was possible to answer several questions relevant to pollen development. One of the first questions asked was: Are the genes expressed in pollen members of large families of genes or are they represented in one or a few copies in the genome? To answer this question Southern hybridization analyses were carried out on restriction endonuclease-digested maize nuclear DNA. The result with one of the clones, Zmc26, is shown in figure 5. This analysis shows that Zmc26 is present in one or a very few copies in the maize genome. Similar analyses with other pollen-expressed clones have shown that all the other clones analysed in this manner are present in one or a few copies in the genome.

EARLY AND LATE GENES

The clones represent mRNAs that are present in mature pollen. We wished to determine when during pollen development the genes are first activated and also the pattern of accumulation of the mRNAs during development. To answer these questions, pollen-specific clones from *T. paludosa* and maize were used as probes in Northern blot hybridizations to RNA isolated from microspores and pollen at various stages of development, beginning with tetrads formed soon after meiosis. The data are shown in figures 6, 7 and 8. The nitrocellulose membrane containing the electrophoretically separated RNAs from the different pollen stages of *Tradescantia* was probed

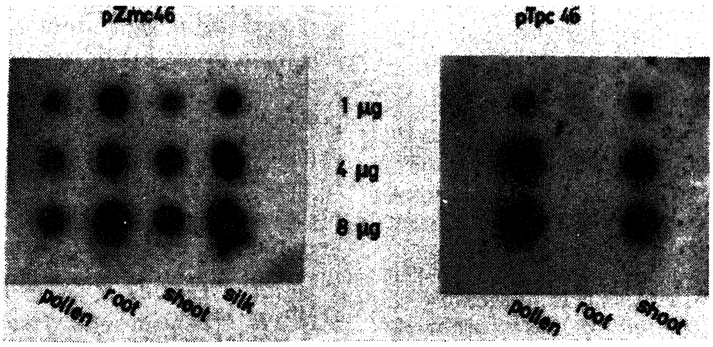

Figure 4. Dot blot analysis of total RNA from different tissues hybridized to ^{32}P-labelled cloned probes that are not pollen-specific: pZmc46 from maize and pTpc46 from *T. paludosa*.

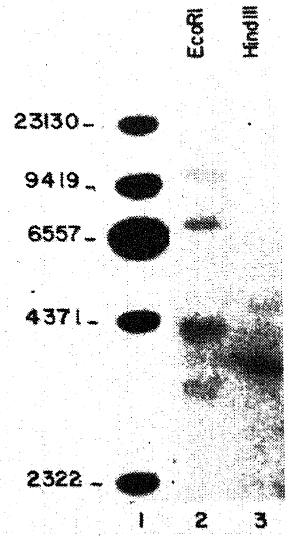

Figure 5. Southern hybridization of restriction endonuclease-digested maize genomic DNA (20 μg per lane) to the pollen-specific clone pZmc26. Lane 1, DNA size markers, in nucleotide pairs; lanes 2 and 3, *Eco*R1 and *Hin*dIII digests respectively.

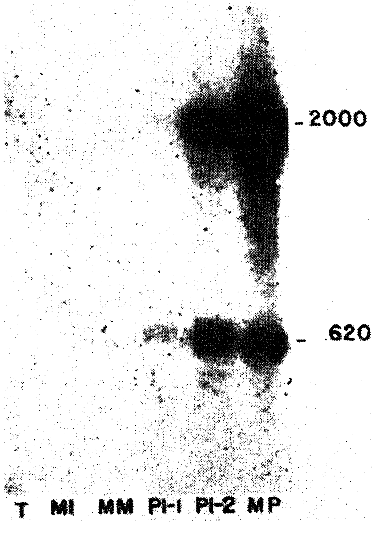

Figure 6. RNA blot hybridization of total RNA (5 μg per lane) from *T. paludosa* pollen at different stages of development to pTpc44 and pTpc70 simultaneously. These clones hybridize to pollen mRNAs of 2000 and 620 nucleotides respectively. T, Tetrads; MI, microspores during microspore interphase; MM, microspores in microspore mitosis: PI-1 and PI-2, pollen at early and late pollen interphase respectively; MP, mature pollen.

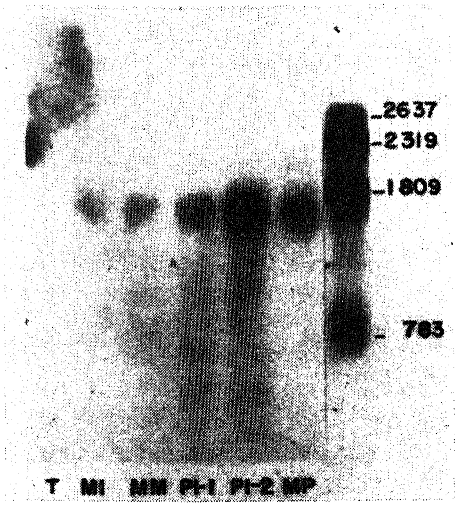

Figure 7. RNA blot hybridizations of total RNA (5 μg per lane) from *Tradescantia* pollen at different stages of development to an actin clone from soybean (pSAc3). The GeneScreen nitrocellulose membrane used in the experiment of figure 6 was stripped of the two probes pTpc44 and pTpc70 and then probed with pSAc3 under conditions of reduced stringency. See figure 6 for legends to the lanes; last lane, cleaved pBR322 size markers.

simultaneously with two pollen-specific clones, Tpc44 and Tpc70 (figure 6). The mRNAs complementary to both these clones are first detectable after microspore mitosis during early pollen interphase, and they continue to accumulate thereafter, reaching their maximum concentration in the mature pollen grain. The nitrocellulose membrane was next stripped of the Tpc44 and Tpc70 probes, and probed with a cloned actin gene from soybean (figure 7). The actin probe hybridizes to an mRNA that is 1750 nucleotides in length. In contrast to the pollen-specific mRNAs, actin mRNA is first detectable during microspore interphase prior to microspore mitosis. It accumulates thereafter, reaching a maximum at late pollen interphase, and then undergoes a substantial decrease in the final stages of maturation of the pollen grain.

A similar pattern of accumulation of mRNAs has also been found for the maize pollen clones (figure 8).

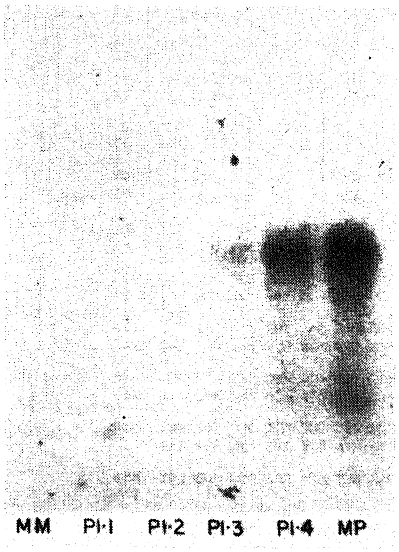

Figure 8. RNA blot hybridization of total RNA (5 μg per lane) from maize pollen at different stages of development using the pollen-specific clone pZmc30 as a probe. Clone pZmc30 hybridizes to an mRNA of 1700 nucleotides. MM, Microspores undergoing microspore mitosis; PI-1 to PI-4, immature pollen grains at different stages of maturity from early through late pollen interphase; MP, mature pollen.

In situ hybridization in tissue sections using labelled RNA transcripts of the pollen-specific clone Zmc13 as probes showed that the Zmc13 mRNA is present in the vegetative cell cytoplasm[10]. It is thus most likely to be a product of transcription of the vegetative nucleus. During early pollen tube growth, the Zmc13 mRNA is found to be distributed throughout the cytoplasm of the pollen tube and is not confined to any particular region of the tube.

All the clones in our libraries that we have tested appear to be 'late'-expressed genes, i.e. they are not activated until after microspore mitosis, which is fairly late in pollen development. Actin is an example of a gene that is first expressed early in pollen development. Alcohol dehydrogenase in maize is another example of a gene expressed early after meiosis[11]. We have recently constructed another library, which represents mRNAs expressed 'early' in pollen development (between meiosis and microspore mitosis), and have begun studies on the 'early' genes. This is necessary for a fuller understanding of the genetic programme required for pollen development.

CURRENT STUDIES

A current major interest of the laboratory is to identify the *cis*-acting elements in the promoter regions of the genes that are responsible for pollen-specific expression. With this goal in mind, we have selected two clones for detailed study. One of the pollen-specific cDNA clones, Zmc13, has been completely sequenced[10] (figure 9). Zmc13 is a full-length cDNA clone 929 nucleotides in length, excluding the poly(A) region. Putative polyadenylation signals are identifiable in the 3′-nontranslated region. The mRNA codes for a predicted polypeptide of 170 amino acid residues and a molecular mass of 18.3 kDa. A conspicuous feature of the hydropathy profile of the predicted polypeptide is a strongly hydrophobic region near the amino terminus, which meets the major criteria for a signal sequence. Hence it is likely that the protein is secreted across a membrane. The function or identity of the protein is at present unknown. A computer search of the National Biomedical Research Foundation Protein Sequence Database and the nucleotide and deduced amino acid GenBank sequences using both the nucleotide and deduced amino acid sequences of Zmc13 has not revealed any meaningful homology with known proteins.

Using the Zmc13 clone as a probe, a genomic library of maize has been screened and the corresponding genomic clone has been isolated. The entire gene has been sequenced, including about 1300 base pairs of nontranscribed 5′-flanking sequence and about 500 base pairs of nontranscribed 3′-flanking sequence. A comparison of the cDNA sequence with the genomic sequence shows that there are no introns in the Zm13 gene.

We are currently attempting to analyse the

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..A GGC CAG ATT TGC CAC CCT CGT CTC ACC CTC CCT CCC TCA CAC AAA TAA TAA GGA AAG   58

GTC CCG CCC TTT TCC TCC GAC ATC CAC AAG GGG GGA GGG GAA AAC ACG TAC ATT CAC CCG  118

GCG GCA ATA ATG GCC TCG GTT CCG GCT CCG GCG ACG ACG ACC GCC GCC GTC ATC CTA TGC  178
            Met Ala Ser Val Pro Ala Pro Ala Thr Thr Thr Ala Ala Val Ile Leu Cys   17

CTA TGC GTC GTC CTC TCC TGT GCC GCG GCT GAC GAC CCG AAC CTC CCC GAC TAC GTC ATC  238
Leu Cys Val Val Leu Ser Cys Ala Ala Ala Asp Asp Pro Asn Leu Pro Asp Tyr Val Ile   37

CAG GGC CGC GTG TAC TGC GAC ACC TGC CGC GCC GGG TTC GTG ACC AAC GTC ACC GAG TAC  298
Gln Gly Arg Val Tyr Cys Asp Thr Cys Arg Ala Gly Phe Val Thr Asn Val Thr Glu Tyr   57

ATC GCG GGC GCC AAG GTG AGG CTG GAG TGC AAG CAC TTC GGC ACC GGC AAG CTC GAG CGC  358
Ile Ala Gly Ala Lys Val Arg Leu Glu Cys Lys His Phe Gly Thr Gly Lys Leu Glu Arg   77

GCC ATC GAC GGG GTC ACC GAC GCG ACC GGC ACC TAC ACG ATC GAG CTC AAG GAC AGC CAC  418
Ala Ile Asp Gly Val Thr Asp Ala Thr Gly Thr Tyr Thr Ile Glu Leu Lys Asp Ser His   97

GAG GAG GAC ATC TGC CAG GTG GTG CTG GTG GCC AGC CCG CGC AAG GAC TGC GAC GAG GTC  478
Glu Glu Asp Ile Cys Gln Val Val Leu Val Ala Ser Pro Arg Lys Asp Cys Asp Glu Val  117

CAG GCG CTC AGG GAC CGC GCC GGC GTC CTG CTC ACC AGG AAC GTT GGC ATC TCC GAC AGC  538
Gln Ala Leu Arg Asp Arg Ala Gly Val Leu Leu Thr Arg Asn Val Gly Ile Ser Asp Ser  137

CTG CGC CCC GCC AAC CCG CTA GGC TAC TTC AAG GAC GTG CCG CTC CCC GTC TGC GCC GCG  598
Leu Arg Pro Ala Asn Pro Leu Gly Tyr Phe Lys Asp Val Pro Leu Pro Val Cys Ala Ala  157

CTG CTC AAG CAG CTG GAC TCG GAC GAC GAC GAC GAC CAG TAA ACT ATA CCA CGG CGG CGT  658
Leu Leu Lys Gln Leu Asp Ser Asp Asp Asp Asp Asp Gln                              170

CGC GGA CAT GCT GCA CAA AAC TAC AAC GAT ACA GAG CGA ACG CAT GGC ATG GAT AGC AGT  718

ATC TAC GGA AAG GAA AGG AAG AAA AGG AAA ATA AAA AAT GTA TCA GAG TGC TTG ATT CAC  778

TTG CTG CTG TCA CCC ATT CCC CGT TCT TAA CAT AAC ATG TGG GCC GGC TTG GCC CAG GCA  838

CAA GCC CAT CTA CGC ATG GCC TAC GGT CCG CTA AAA TAT AGC CCT AAT TAT GAG CCG TGT  898

TGT GCC GTC ACA TGG ATC GAT CCA GCG GCA TAA AAA AAA AAA AAA ...                  929
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Figure 9. Nucleotide sequence of the cDNA clone Zmc13 and its predicted amino acid sequence. Underlined sequences indicate possible polyadenylation signals. (Reprinted with permission from ref. 10.)

promoter region by deletion mutagenesis and transformation to identify the sequence elements necessary for pollen-specific expression of the Zm13 gene.

SUMMARY

A large number of genes are expressed in pollen. Most of these genes are also expressed in vegetative tissues and only a small fraction, probably about 10%, are pollen-specific. There appear to be at least two classes of genes based on the stage in pollen development when the genes are first activated. Genes such as those for actin and Adh1 are turned on soon after meiosis and their transcripts reach a maximum before the pollen grain is fully mature. These 'early' genes probably have primary functions in early pollen development. Other genes, such as several in our cloned cDNA libraries, are first activated after microspore mitosis and the mRNAs then accumulate, reaching maximum concentration in the mature pollen grain at anthesis. This would suggest that these 'late' genes have functions late in pollen development and/or during pollen germination and early tube growth. The site of synthesis of one pollen-specific gene, Zm13, is the vegetative cell of the pollen grain. The entire nucleotide sequence of Zm13 cDNA and that of the corresponding genomic DNA have been determined. The promoter region of this gene is currently being analysed with the goal of identifying *cis*-acting sequence elements required for pollen specificity of expression of the gene.

ACKNOWLEDGEMENT

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10 June 1989

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ANNOUNCEMENT

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EPOXIDATION OF CHALCONES WITH *t*-BUTYL HYDROPEROXIDE IN PRESENCE OF AN IRON(III) SCHIFF BASE COMPLEX

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ABSTRACT

Epoxidation of chalcone, 4-methoxy-, 4′-chloro-, 4′-bromo-, 3′-nitro-, 4′-methyl-3-nitro-, and 3′-nitro-3-nitrochalcones has been carried out with *t*-butyl hydroperoxide using *N*, *N*′-ethylenebis(salicylideneiminato)iron(III)–μ-oxo-*N*, *N*′-ethylenebis(salicylideneiminato)iron(III) complex as a catalyst. The effects of solvents, temperature, quantity of catalyst and different substituents on the yield of epoxides have also been studied. A probable free radical mechanism has been proposed.

INTRODUCTION

SIGNIFICANT amount of work has been done on the preparation, characterization and industrial applications of epoxides[1–4]. Information concerning the role of metal complexes in oxidation has been gained from studies of their reactions with alkyl hydroperoxides under nonautoxidizing conditions[5–7]. *t*-Butyl hydroperoxide (TBHP) is thermally more stable and is less sensitive to contamination by metals than either organic peracids or hydrogen peroxide and is, therefore, safer to handle[8, 9]. The present communication deals with the epoxidation reaction of chalcones with TBHP in presence of (Fe salen)$_2$O complex as a catalyst.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin–Elmer 399 IR spectrometer and UV-visible spectra on a Beckman Du-6, UV/vis spectrophotometer. ^{1}H NMR and mass spectra were recorded on a Varian 80-FT and spectra were obtained from a Jeal JMS–D mass spectrometer. Homogeneity and purity of the products were checked by TLC.

All reagents used were of analytical grade. Solvents were distilled and dried before use. Melting points are uncorrected. The *N*, *N*′-ethylenebis(salicylideneiminato)iron(III)–μ-oxo-*N*, *N*′-ethylenebis(salicylideneiminato)iron(III) complex was synthesized by reported methods[10].

Synthesis of substrates

Various substrates (1a–1g) were synthesized following reported methods[11]. Chalcone (1a), 4-methoxychalcone (1b), 4′-chlorochalcone (1c), 4′-bromochalcone (1d), 3′-nitrochalcone (1e), 4′-methyl-3-nitrochalcone (1f) and 3′-nitro-3-nitrochalcone (1g) had melting points 56, 61, 138, 119, 145, 230 and 158° respectively. μ(KBr) 1645–1695 (–C=O), 1610–1630 (–C=C–) and 965–985 cm^{-1} (–C–H–); λ_{max}(EtOH) 251–309 nm.

Epoxidation of substrates (1a–1g)

Chalcone (2.4 mmol), benzene (50 ml), catalyst (0.5 mmol), 10% aqueous NaOH (5 ml) and 80% TBHP (5 ml) were stirred at room temperature for 24–50 h. The TBHP and aqueous NaOH were added after 26 and 40 h. The reaction was monitored by TLC. The catalyst was filtered off and washed with CH_2Cl_2. The organic layer was separated off, washed with H_2O, 10% Na_2SO_3 and H_2O, and then dried. After evaporation of the solvent, the crude product was eluted on a silica gel column using CH_2Cl_2 as eluant. The product thus obtained was crystallized from methanol.

RESULTS AND DISCUSSION

The rate of epoxidation of substrates (1a–1g) is enhanced by electron donating groups as they elevate the energy of the π bond (HOMO), whereas electron withdrawing groups decrease the same and retard the rate of epoxidation (table 1). Most of the substrates under study have an electron withdrawing group and so the olefinic bond has lower electron density than in the unsubstituted chalcone. These substrates particularly do not undergo epoxidation in the absence of the catalyst. Thus (Fe salen)$_2$O tends to improve the yield of the epoxides of electron-poor olefins. In the epoxidation process, pH also plays an important role. For example, 4-

Table 1 *Epoxidation of chalcones with TBHP and (Fe salen)$_2$O carried out at room temperature in benzene*

	Substrate	Time (h)	Yield (%)
1a	Chalcone	30	79
1b	4-Methoxychalcone	24	82
1c	4′-Chlorochalcone	40	42
1d	4′-Bromochalcone	36	51
1e	3′-Nitrochalcone	35	60
1f	4′-Methyl-3-nitrochalcone	40	70
1g	3′-Nitro-3-nitrochalcone	38	35

methoxychalcone on epoxidation afforded the epoxide in 82% yield when the pH of the reactants was maintained between 7 and 8 (table 2).

The solvent also affects the yield of the epoxides. From table 3, it is evident that the rate decreases with decrease in the dielectric constant of the solvent.

The low yield of the epoxides at high temperatures is probably due to the decomposition of the oxidizing agent. The most suitable temperature for the reaction was found to be ~22°C (table 4).

In the absence of the catalyst, epoxides were obtained in negligible amounts. The optimum substrate : catalyst ratio has been found to be $2.4 \times 10^{-3} : 0.5 \times 10^{-3}$. As the amount of catalyst is decreased, the rate of epoxidation also decreases (table 5).

The epoxidation of chalcones using TBHP, diethyl tartrate and iron(III) schiff base complex was carried out following Sharpless oxidation method[12]. However, no asymmetric induction was observed in these cases.

The IR spectra of the epoxides show the characteristic bands of the oxirane ring in the range 1220–1255 cm^{-1} and those of the carbonyl group at 1650–1690 cm^{-1}. The ^{1}H NMR spectra in CDCl$_3$ show characteristic doublets at δ 4.40–4.67 and 4.03–4.36 ppm for three-membered ring protons.

In order to finally ascertain whether free radicals

Table 2 *pH Dependence of the epoxidation of chalcones at room temperature*

Chalcone			4-Methoxy-chalcone			4′-Chloro-chalcone			4′-Bromo-chalcone			3′-Nitro-chalcone			4′-Methyl-3-nitrochalcone			3′-Nitro-3-nitrochalcone		
pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)	pH	Time (h)	Yield (%)
5.0	40	0	5.0	40	0	5.0	50	0	5.0	40	0	5.0	45	0	5.0	30	0	5.0	40	0
6.0	38	25	6.0	38	35	6.2	45	10	6.1	45	18	6.5	40	20	6.0	35	22	6.0	42	7
7.5	30	79	7.8	24	82	7.6	40	42	7.9	36	51	7.7	35	60	7.8	40	70	7.5	38	35
9.3	30	0	9.3	30	0	9.0	40	0	9.1	35	0	9.2	45	0	9.3	45	0	9.0	50	0

Table 3 *Solvent effect on the epoxidation of chalcones at room temperature*

		Chalcone		4-Methoxy-chalcone		4′-Chloro-chalcone		4′-Bromo-chalcone		3′-Nitro-chalcone		4′-Methyl-3-nitrochalcone		3′-Nitro-3-chalcone	
Solvent	Dielectric constant	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
Dichloromethane	9.08	30	83	20	88	35	55	38	58	36	65	35	76	40	42
Benzene	2.284	30	79	24	82	40	42	36	51	35	60	40	70	38	35
Carbon tetrachloride	2.238	35	75	35	80	35	39	35	42	38	52	45	65	35	80
Cyclohexene	2.220	38	67	40	70	35	32	40	38	38	45	40	51	40	25

Table 4 *Temperature effects on the epoxidation of chalcones*

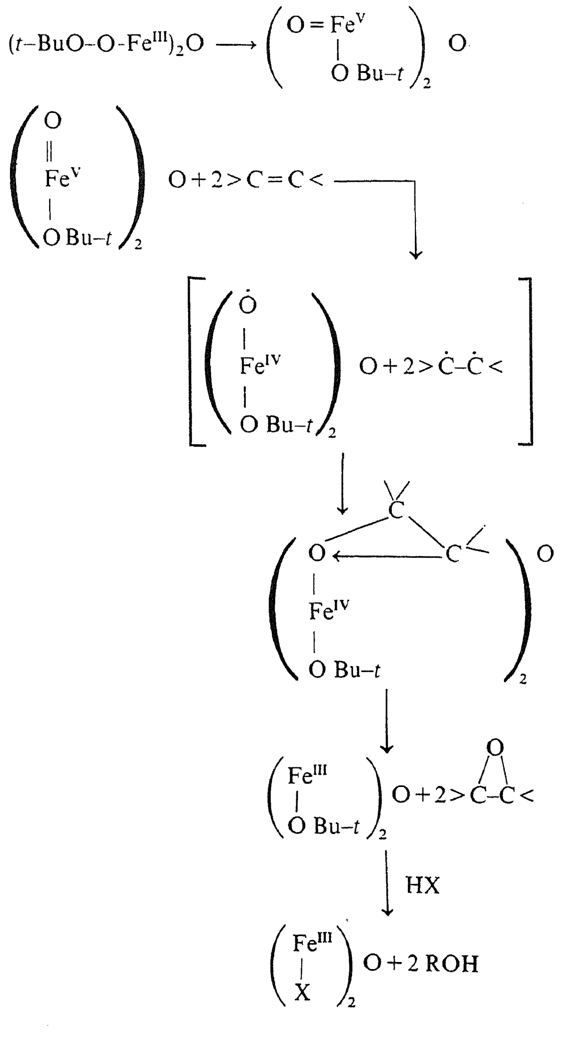
	Chalcone		4-Methoxy-chalcone		4′-Chloro-chalcone		4′-Bromo-chalcone		3′-Nitro-chalcone		4′-Methyl-3-nitrochalcone		3′-Nitro-3-nitrochalcone	
Temperature (°C)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
0	85	46	80	52	80	30	90	35	80	38	82	40	80	15
25	30	79	24	82	40	42	36	51	35	60	40	70	38	35
40	55	20	50	33	50	8	50	15	50	18	50	19	50	5
60	64	15	55	20	60	5	60	10	62	12	60	14	60	0

Table 5 *Effect of substrate : catalyst ratio on the rate of epoxidation of chalcones at room temperature*

		Chalcone		4-Methoxy-chalcone		4′-Chloro-chalcone		4′-Bromo-chalcone		3′-Nitro-chalcone		4′-Methyl-3-nitrochalcone		3′-Nitro-3-nitrochalcone	
Substrate (mmol)	Catalyst (mmol)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)
4.8	0.5	35	75	28	78	42	40	40	45	35	50	35	62	40	25
2.4	0.5	30	79	24	82	40	42	36	51	35	60	40	70	38	35
2.4	0.2	35	32	35	40	40	18	42	20	40	25	40	28	45	10
2.4	0.4	32	45	30	55	38	20	40	24	40	30	35	33	40	12

play any role in these reactions, the effect of radical traps was studied on adding diphenylpicrylhydrazyl, a well-known radical scavenger, to the reacting system. The rate of the reaction was observed to decrease. This amply proves that these reactions involve a radical mechanism.

$$(X\,Fe^{III})_2O + t\text{-}BuO_2H \longrightarrow (t\text{-}BuO\text{-}O\text{-}Fe^{III})_2O + HX$$



Scheme 1

Thus homolytic catalysis occurring in these reactions involves a one-electron process, in which free radicals are intermediates. Rapid decomposition of TBHP occurs in presence of trace amounts of the iron catalyst. A mechanism for this process of epoxidation can be proposed (scheme 1), involving the formation of an oxorion(V) species, which is an active oxidant. This participates in a 1,2-addition to the olefinic double bond.

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ELECTRON DONOR–ACCEPTOR COMPLEXES OF PHENOTHIAZINE WITH IODINE

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ABSTRACT

Electron donor–acceptor complex formation between phenothiazine (PTZ) and iodine (I_2) both in solid state and in solution are investigated by electronic, IR and high-resolution (270 MHz) FT-proton NMR spectroscopy, thermogravimetric analysis, differential thermal analysis, and mass spectral studies. PTZ forms PTZ–I_2, PTZ–$2I_2$, PTZ–$3I_2$ and PTZ–$4I_2$ charge-transfer complexes with iodine in solution. The study indicated that it forms only one type of complex, PTZ–I_2, in solid state with stoichiometry 1 : 1. The charge transfer interaction between PTZ and I_2 seems to be very strong. The mode of interaction appears to depend on the type of electron acceptor and the chemical environment.

INTRODUCTION

GUTMANN and Keyzer[1] noted the intensity of the ESR signal of the powdered phenothiazine-iodine system, which rose with increase in iodine content to a maximum at 2:3 ratio. It was also observed that the signal intensity at 2:3 ratio was twice as strong as that at 1:1 and a little stronger than that at 1:2. These authors, on the basis of conductivity, IR and ESR spectroscopic studies, concluded that at least three different species of complex between PTZ and I_2 are likely to exist. They have also reported that the composition 2:3 is the stable one with the probable structure I_2^-–PTZ^+. I_2. PTZ^+. I_2^-. Matasunga[2], on the basis of IR measurements, reported that the PTZ–I_2 complex is dative in the ground state. Bhat and Rao[3] studied the solid charge-transfer (CT) complexes of PTZ–I_2 in the ratios 1:1, 1:3 and 3:1. This system was found to show change in sign of the majority charge carriers with variation in donor–acceptor ratio. Chan *et al.*[4] prepared PTZ–I_2 complex by grinding PTZ and I_2 together in 1:2 molar ratio and reported that the complex is generally dative in the ground state. Perissinotti *et al.*[5] studied galvanic formation cells with β-silver iodide as electrolyte and mixtures of PTZ–I_2 of different compositions as cathode. From the observation of EMF plateaus of the galvanic cells they determined the existence and the limits of the four domains in the PTZ–I_2 system. These domains correspond to the two stoichiometric ones $(PTZ)_2$–I_2 and $(PTZ)_2$–I_6, and two non-stoichiometric ones, (PTZ)–I_{5-x} and (PTZ)–I_{7-x}. Saksena and Aradhana[6] measured the dielectric constants of various compositions of the PTZ–I_2 system in benzene at a radio frequency of 1 MHz at 30 and 40°C. Kinetic and thermodynamic parameters indicate a 1:1 molar association between PTZ and I_2. A literature survey reveals that studies of the CT interaction between PTZ and I_2 are scanty. It is felt that the nature of interaction between PTZ and I_2 needs some further investigation. The present study of the CT interaction of PTZ with I_2 using electronic, IR and high-resolution FT–NMR spectroscopy and thermo-analytical techniques seems to throw more light on the pharmacological action of the phenothiazine drugs.

EXPERIMENTAL

A commercially available sample of phenothiazine (Fluka AG, Switzerland) was purified by repeated crystallization from double-distilled benzene and dried over P_2O_5 under vacuum; m.p. 182°C. Iodine, BDH Analar sample, was used as received. All other reagents were of Analar grade.

A Beckman model DB spectrophotometer with 1 cm matched silica cells was used for electronic spectra. A Perkin-Elmer IR model 580 was employed to record IR spectra using KBr pellet technique. A Bruker WH-270 pulsed FT ^{1}H-NMR spectrometer was used to obtain ^{1}H-NMR spectra. Thermo-analytical studies were carried out using a Stanton Redcroft DTA 674 instrument. Mass spectra were recorded using a Hewlett–Packard 5995 mass spectrometer.

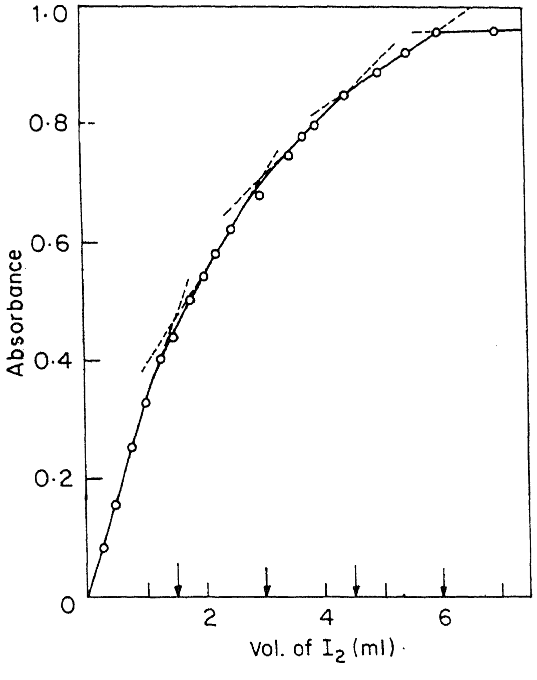

Figure 1. Mole ratio plot for phenothiazine–iodine system.

PROCEDURES

PTZ–I_2: Mole ratio method

Exactly 1.5 ml portions of 2×10^{-3} M PTZ in ethyl alcohol were transferred into 20 separate 10 ml standard flasks. Then 0.5, 1.0, 1.5, . . . , 6.5 and 7 ml of 2×10^{-3} M iodine in ethyl alcohol were transferred to the above flasks. The solutions were made up with ethyl alcohol and mixed for uniform concentration. Percentage transmittance was recorded at the wavelength of maximum absorption λ_{max} 650 nm. A graph of absorbance vs volume of iodine added was drawn to obtain the mole ratio curve. The linear portions of the curve were extrapolated to determine the compositions of various species of the complex (figure 1).

Preparation of solid complexes

PTZ (0.5 g) in 50 ml ethyl alcohol solution was mixed with 150 ml of a solution of 0.64 g of I_2 in ethyl alcohol. The solution was stirred for 2–3 h. The solid complex formed (PTZ–I_2) was filtered using a G4 sintered glass crucible and washed with carbon tetrachloride. The complex was dried over P_2O_5 in a vacuum desiccator.

PTZ–$2I_2$, PTZ–$3I_2$ and PTZ–$4I_2$ were prepared as above using 1.28, 1.95 and 2.6 g of I_2 in 150 ml ethyl alcohol respectively.

RESULTS AND DISCUSSION

The electronic spectra of PTZ, I_2 and PTZ–I_2 (1:1) were recorded in ethyl alcohol and are shown in figure 2 (see also table 1). There is no marked

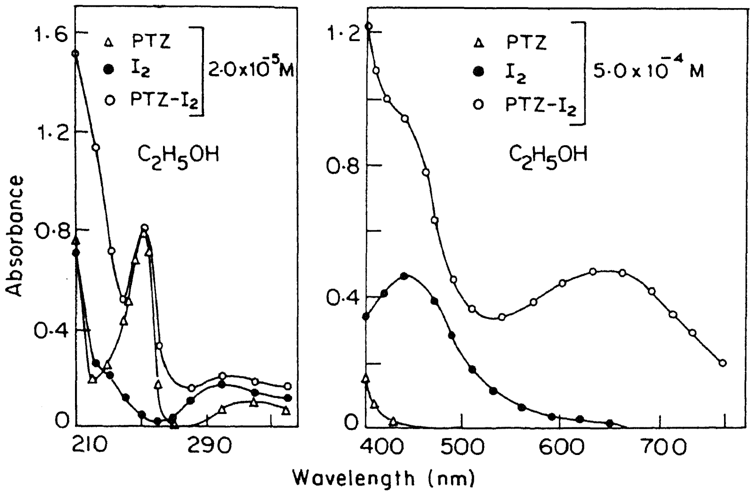

Figure 2. Electronic spectra (UV and visible) of phenothiazine, iodine and phenothiazine–iodine complex.

Table 1 *UV-visible absorption data (in ethyl alcohol) for PTZ, I_2 and PTZ–I_2*

	$\lambda_{max}(\varepsilon, l\,mol^{-1}\,cm^{-1})$	
Compound	Ultraviolet	Visible
PTZ	225.0 *sh* (1.05×10^4)	
	252.0 (2.68×10^4)	
	318.0 (4.36×10^3)	
I_2	225.0 *sh* (1.20×10^4)	
	298.0 (9.35×10^3)	440.0 (9.5×10^2)
PTZ–I_2	225.0 *sh* (4.68×10^4)	430.0 *sh* (3.88×10^3)
	250.0 (3.90×10^4)	634.0 (1.92×10^3)
	304.0 (1.05×10^4)	

sh, shoulder.

difference in the UV spectrum of PTZ–I_2 complex in comparison to the summation spectrum of PTZ and I_2 except that an increased intensity of the shoulder at 225 nm is observed in the complex. In the visible region the complex shows a shoulder with higher intensity corresponding to the absorption of I_2 at 440 nm with a slight blue shift (~10 nm); in addition, a broad band at 634 nm is observed in the spectrum of the complex, which may be assigned to the CT band. The absorption at 440 nm in the I_2 spectrum may be assigned to the transition ... $(\sigma_g 5P)^2 (\pi_u 5P)^4 (\pi_g 5P)^4 \rightarrow \ldots (\sigma_g 5P)^2 (\pi_u 5P)^4 (\pi_g 5P)^3 (\sigma_u 5P)$. This transition has been found to be slightly affected in the complex. Investigations of the stoichiometry of the PTZ and I_2 complex using the principle of the mole ratio method[7] indicated that there are four types of complexes in solution with the stoichiometry 1:1, 1:2, 1:3 and 1:4 (PTZ–I_2, PTZ–$2I_2$, PTZ–$3I_2$ and PTZ–$4I_2$).

The IR spectra of the CT complex of PTZ–I_2 along with the spectrum of PTZ are presented in figure 3. The IR spectra corresponding to the ratios 1:1, 1:2, 1:3 and 1:4 (PTZ–nI_2) are identical in appearance. Remarkable changes are observed in the spectra of the complexes in comparison with the spectrum of PTZ. The new intense vibrational bands observed are 1070, 1110, 1335 and 1395 cm^{-1}, which indicate strong interaction between PTZ and I_2. The N–H stretching frequency at 3330 cm^{-1} of PTZ is absent in the spectra of the complexes. The bands around 1300–1310 cm^{-1} due to C–N stretching and the C–H out-of-plane bending around 710–748 cm^{-1} are shifted to higher energy regions in the spectra of the complexes. The IR spectra of the complexes show strong CT interaction between PTZ and I_2, which seems to affect the N–H stretching frequency of the electron system of the PTZ molecule.

Proton NMR spectra of PTZ, PTZ–I_2, PTZ–$2I_2$, PTZ–$3I_2$ and PTZ–$4I_2$ were recorded in DMSO-d_6. All the four compounds gave qualitatively identical NMR spectra. The data indicate that PTZ and I_2 form only one type of complex corresponding to the composition 1:1 in the solid state unlike in solution. High-resolution proton NMR spectra of PTZ and PTZ–I_2 are shown in figure 4. The N–H proton of PTZ undergoes shielding in the process of CT interaction between PTZ and I_2. All the aromatic protons 1, 2, 3, 4, 6, 7, 8 and 9 ($\delta = 6.6665$–7.0185 ppm) of PTZ are deshielded in the spectra of the complexes. The signals corresponding to these protons appear in the range 7.5819–7.0794 ppm. Greater hyperfine structure is observed for the aromatic protons of PTZ. This may be due to the presence of long-range coupling and the different influences of the S and N

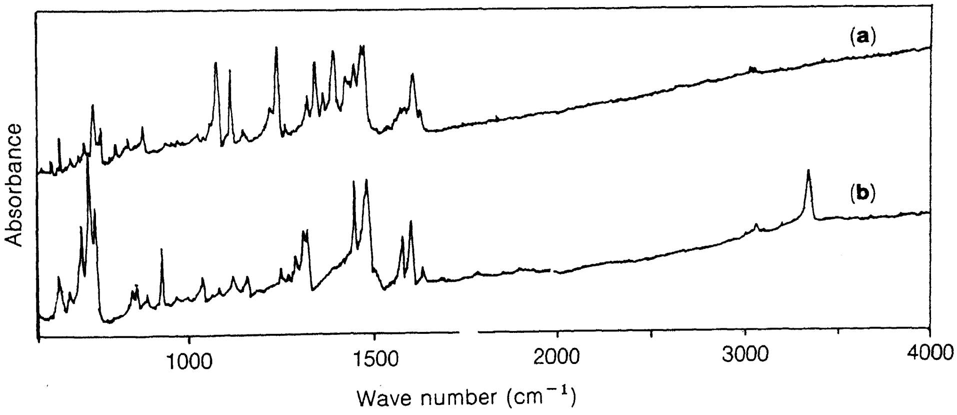

Figure 3. IR spectra of (**a**), phenothiazine and (**b**), phenothiazine–iodine complex.

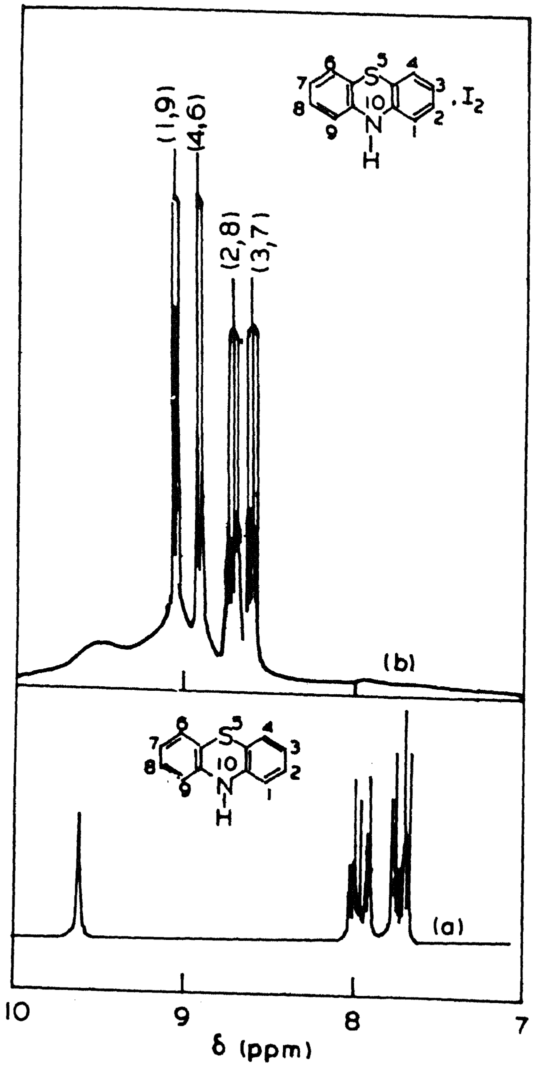

Figure 4. High-resolution (270 MHz) proton FT-NMR spectra of (**a**), phenothiazine and (**b**), phenothiazine–(iodine)$_2$ complex.

atoms at positions 5 and 10 on the protons (4, 6) and (1, 9). The CT interaction between PTZ and I_2 seems to make the above effects less on the chemical environment of the protons 1, 4, 6 and 9, with the result that simpler splittings corresponding to the aromatic protons of PTZ are observed in the complex. The two doublets at 8.0483–8.0777 ppm and 7.9068–7.9353 ppm may be assigned to the protons (1, 9) and (4, 6) of the PTZ–I_2 complex respectively. The two triplets at 7.6976–7.7529 ppm and 7.5838–7.6494 ppm may be assigned to the protons (2, 8) and (3, 7) of the complex respectively. The assignments seem to be confirmed by the ortho coupling constant values (9–10 Hz). The long-range couplings are absent in the complex.

The thermogravimetric analysis curves, mass spectral data and differential thermal analysis (DTA) curves also support the above conclusions regarding the stoichiometry. The complex starts to degrade at 200°C and is found to lose weight of about 60% over the temperature range 200–500°C. The complex degrades in two steps. The first weight loss may be due to the loss of iodine and the second weight loss could be due to loss of iodine as well as degradation of the PTZ structure. The DTA curve of complex PTZ–I_2 shows a sharp endothermic peak at 232°C due to the melting. The compound seems to be stable up to the melting point and decomposes above this temperature. A mass spectral study at 200°C of the PTZ–I_2 complexes gave identical mass spectra. The *m/e* peaks from 40 to 800 have been scanned. Only two peaks are observed, the base peak *m/e* = 127 and another peak *m/e* = 254 with 60% intensity of the base peak. These peaks are due to atomic iodine and molecular iodine respectively.

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SHORT COMMUNICATIONS

EXAMPLES OF SHELL PREDATION BY PREDATORY GASTROPODS AND PARASITES IN THE MIDDLE EOCENE ROCKS OF KUTCH, GUJARAT

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SOME carnivorous species have the capacity to penetrate and drill holes in the calcareous exosketelons of invertebrates. Interesting observations of such activity were made on the highly calcified oyster shells recovered from the Tertiary rocks of Kutch near Lakhpat, Gujarat (23° 44′ N, 68° 50′ 01″ E). The oyster specimens were collected from a 2-metre-thick exposed section of dirty white monotonous cream and dull yellow foraminiferal limestone. Occurrence of *Nummulites beaumonties*, *N. pinfoldi*, *Discocyclina dispansa*, *Orbulinoides beckmanni*, etc. confirm its Middle Eocene age. Lithological and micropalaeontological evidence indicates littoral to sublittoral shallow marine environment.

Bore hole morphology

Borehole morphology is typically developed on the larger or the left valve of the oyster shells, and includes prominent vertical cylindrical boring and horizontal scratched groove tunnels. The vertical boreholes are found in two size groups. The larger bores have a maximum depth of 0.45 cm and diameter of 0.6 cm, giving a depth/diameter ratio of 0.75. The smaller bores, which are more abundant, have maximum depth of 0.30 cm and diameter <0.1 cm (depth/diameter ratio 3.0). The vertical boreholes have sharp edges, flat and rough bottom, smooth wall and axis of boring at right angles to the shell surface (figure 1).

The horizontal scratched open groove structures are in the plane of the surface of the shell. Very often these structures connect the vertical unfinished boreholes. The maximum length observed of such a groove is 10 cm, with depth 0.35 cm and width about 0.5 cm. The edges of the two parallel sides are sharp, the sides rough-walled, and the bottom broad and irregular.

Borings, interpreted as biogenic sedimentary structures, indicate behavioural patterns of the organisms in direct response to their life functions. The boreholes in oysters reported here indicate two different boring organisms; the behaviour is believed to be predatory in one case and parasitic in the other. Two different organisms are suspected because of the distribution of two different borehole morphologies. A predatory habit is inferred for the vertical cylindrical bores as this morphology shows that the originator, in search of food, attempted to gain entrance into the bivalve oyster shell rather than bore for its domicile. Four facts support the suggestion that the predatory organisms were gastropods—the size of the hole, the presence of more than one hole, preference of the larger, left valve of the oyster shell, and typical vertical drilling as adopted by present-day carnivorous gastropods[1-5].

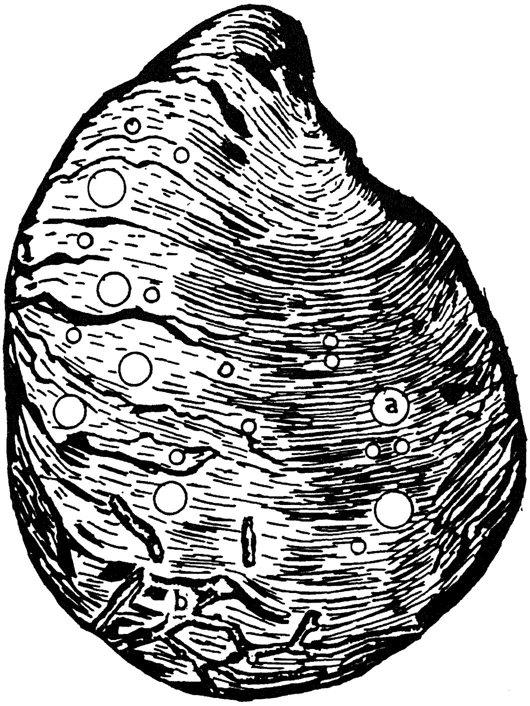

Figure 1. Drawing of oyster shell showing the two types of borings. (a) Vertical cylindrical boring made by predatory gastropods, (b) horizontal tunnels made by parasitic worms.

Cadee[5] distinguishes these gastropods as drillers, chippers and crushers. In the case of our described specimens, we suspect driller gastropods.

According to Keir[6], many snails and parasitic worms have the capacity to excavate tunnels through the shell surface in a short time. The architecture of the tunnels in our specimens rule out the possibility of parasitic gastropods. The preferential location of the tunnels near the posterior end of the oyster shells rather indicates the action of parasitic worms. These worms possibly fed upon the waste brought out through the excurrent siphons and lived a symbiotic life with the oysters. It is further thought that the tunnels were possibly used by the worms as their protective domiciles. Finally it could be argued that the prey (oysters), the predators (gastropods) and the parasitic animals (worms) coexisted in the shallow marine habitat during the Middle Eocene when the limestones in Lakhpat were being deposited.

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ORTHOHYDROXYACETOPHENONE THIOSEMICARBAZONE AS A NEW ANALYTICAL REAGENT FOR THE SPECTROPHOTOMETRIC DETERMINATION OF COPPER

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THIOSEMICARBAZONES have been frequently employed as chromogenic reagents for the spectrophotometric determination of inorganic ions and their analytical potentialities have been reviewed[1,2]. Here we describe the synthesis of *o*-hydroxyacetophenone thiosemicarbazone (OHAPTS) and its use as analytical reagent for the determination of copper. Most of the spectrophotometric methods reported in the literature suffer from many disadvantages—low sensitivity, heating, extraction, and tolerance limit of associated ions[3-7]. The proposed method, besides being sensitive, is simple and rapid, requires no heating or extraction, and has the advantage of virtual freedom from interference of many associated foreign ions. The high tolerance limit of zinc in presence of citrate allows the method to be used for the determination of copper in zinc-based alloys and steel samples.

The reagent was prepared by a procedure similar to that of Aydin[8] and Reddy[9] (yield 65%, m.p. 183–186°C). The reagent (0.5232 g) was dissolved in 50 ml of DMF (5×10^{-2} M). Solutions of lower concentration were obtained by dilution with DMF. Stock solution of Cu(II) was prepared by dissolving 2.4969 g of copper sulphate pentahydrate (BDH, AR) and standardized titrimetrically[10]. All chemicals used were of BDH AR grade.

To each of a set of 25 ml volumetric flasks containing 12.5 ml of buffer (acetic acid–sodium acetate) of pH 5.5, 3 ml of DMF, 2 ml of reagent (5×10^{-2} M) and an aliquot of copper(II) solution (5×10^{-4} M) were added and made up with distilled water. The absorbance at 360 nm of each solution was measured against reagent blank. A calibration graph was drawn for the relationship between the amount of copper(II) and the absorbance.

The optimum pH range for maximum colour development was 5.5–8.0. Hence sodium acetate–acetic acid buffer of pH 5.5 was selected for further studies. A five-fold excess of the reagent is sufficient for getting maximum and constant absorbance. The order of addition of constituents (buffer, DMF, metal, reagent) has no effect on the absorbance.

A linear graph is obtained for absorbance vs amount of metal ion in the range 0.42 to 6.6 μg ml^{-1} of copper(II). The molar absorptivity and Sandell's sensitivity of the method are 9.5×10^{3} l mol^{-1} cm^{-1} and 0.00669 μg cm^{-2} respectively. The standard deviation for 10 determinations of 64 μg of copper(II) was ±0.0006. The practical range of determination of copper(II) as envisaged by Ringbom's plot was 0.67 to 3.94 μg ml^{-1}.

Vosburgh and Cooper's[11] method indicates the presence of one complex species in solution. Also Job's and molar ratio and slope ratio methods show the presence of 2:3 (M:L) species. The stability constant of the complex is 6.60×10^{12}.

Several foreign ions were examined for their effect on copper(II) in the recommended procedure for the spectrophotometric determination of copper. An error of ±2% in absorbance was considered to be tolerable. The tolerance limit (in μg) for various ions in the determination of 48 μg of Cu(II) at pH 5.5 is as follows:

Pb(II), 3100; W(VI), 2760; Al(III), 1620; Mo(VI), 1440; Zr(IV), 1370; Th(IV), 1160; Cd(II), 565; Mn(II), 560; Ti(II), 480; Mg(II), 390; Ce(IV), 280; Au(III), 110; V(IV), 100; Co(II), 58; citrate, 40,000; tartrate, 34,800; iodide, 19,350; nitrate, 18,600; thiocyanate, 17,400; sulphate, 14,400; bromide, 11,950; chloride, 10,635; phosphate, 9797; fluoride 5700; oxalate, 4400. However, Ni(II), Zn(II) and Cr(III) above 300, 1500 and 300 μg respectively interfere and can be masked

Table 1 *Determination of copper in alloy steels*

Steel sample	Copper (μg) Taken	Found	Error (%)
BCS 409	17.25	17.10	0.87
	23.00	22.70	1.30
	28.75	28.60	0.52
BCS 406-I	21.00	20.90	0.48
	28.00	27.90	0.35
	35.00	34.50	1.43
BCS 406	24.00	23.60	1.67
	32.00	31.60	1.25
	40.00	39.70	0.75
SRM 94-C	50.50	49.52	1.98
	75.75	75.50	0.35
	101.00	100.75	0.25

	Content (%) Mn	Cr	Mo	Ni	Cu	V	Co	Al	Mg	Fe	Pb	Cd
BCS 409	0.48	1.22	0.77	3.14	0.23	0.280	—	—	—	—	—	—
BCS 406-I	0.61	2.10	1.00	1.52	0.28	0.017	0.006	—	—	—	—	—
BCS 406	0.53	2.12	1.03	1.69	0.32	0.084	0.047	—	—	—	—	—
SRM 94-C	0.014	—	—	0.006	1.01	—	—	4.07	0.042	0.0018	0.06	0.002

Table 2 *Comparison of OHAPTS with other thiosemicarbazone reagents*

Reagent	Molar absorptivity (l mol^{-1} cm^{-1})
Bipyridylglyoxal bis(4-phenyl-3-thiosemicarbazone)	9200
2,2′-Dihydroxybenzophenone thiosemicarbazone	8600
3-Hydroxypicolinaldehyde thiosemicarbazone	8500
Acetophenone thiosemicarbazone	8300
Glyoxal dithiosemicarbazone	6700
p-Anisaldehyde thiosemicarbazone	6100
Acenapthoquinone thiosemicarbazone	5800
1,2-Cyclohexanedione bisthiosemicarbazone	5700
1,3-Cyclohexanedione bisthiosemicarbazone monohydrochloride	5000
2-Hydroxyiminocyclohexanone thiosemicarbazone	5530
Salicylaldehyde thiosemicarbazone	4800
1,4-Cyclohexanedione bisthiosemicarbazone	4100
Cyclohexane thiosemicarbazone	1300
Orthohydroxyacetophenone thiosemicarbazone	9500 (present work)

with citrate up to 1000, 20,000 and 1000 μg respectively. Fe(III) (6100 μg) can be eliminated by the addition of phosphate. Ag(I) and Sn(II) interfere seriously.

In order to confirm the usefulness of the proposed spectrophotometric method, it was applied to the determination of copper in zinc-based steel samples of BCS 406, BCS 406-1, BCS 409 and SMR 94-C type (nickel, chromium and zinc masked with citrate). The results are summarized in table 1.

The reagent OHAPTS was compared with some well-known thiosemicarbazones[2,12] (table 2). It was found that OHAPTS is a sensitive reagent.

Thanks are due to Dr L. D. Prabhakar, SV Engineering College, Madras, for his interest in this work.

9 September 1988

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SYNTHESIS AND STRUCTURAL STUDIES ON THE COMPLEX OF COPPER(II) WITH *N*-(*p*-TOLYL)-*N*′-BENZOYL THIOCARBAMIDE

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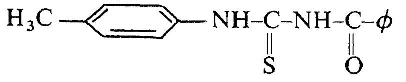
N-(*p*-Tolyl)-*N*′-benzoyl thiocarbamide (TBTC) is a good coordinating agent[1]. The present communication reports interaction of copper(II) with TBTC. TBTC has been given the following structure



All the chemicals used were of BDH or equivalent grade. The ligand (TBTC) was prepared as reported in the literature[2]. Conductance measurements were carried out on a 10^{-3} M solution of the complex. The complex was analysed for metal content using standard procedures[3]. Sulphur was determined as $BaSO_4$ and carbon, hydrogen and nitrogen microanalytically. Magnetic susceptibility measurement was made at room temperature on a Cahn–Faraday electrobalance using $Hg[CO(NCS)_4]$ as a calibrant; experimental magnetic susceptibility was corrected for diamagnetism[4]. The IR spectra of the ligand and the complex were recorded on a Perkin–Elmer IR spectrometer Model-621 in nujol (4000–200 cm^{-1}). The electronic spectra of the complex were recorded on a Carry-14 spectrophotometer in nujol mull. The polycrystalline ESR spectrum of the complex at room temperature was recorded on an E-line X-band spectrometer using TCNE as *g* marker. Antifungal activity of the complex and the ligand was determined at different concentrations as described by Schmitz[5].

The complex was prepared by slowly adding an aqueous ethanolic solution of cupric acetate in the presence of 1–2 drops of glacial acetic acid to a solution of TBTC in acetone in 1:2 molar ratio with constant stirring. A yellow precipitate was formed immediately. It was filtered, washed several times with water, ethanol, acetone and finally with ether, and dried *in vacuo*.

Copper(II)–TBTC complex (m.p. > 300°C) is yellow and is insoluble in water, acetone, ethanol and methanol but soluble in DMF. The molar conductance of the complex in DMF is 26 ohm^{-1} mol^{-1} cm^2, showing that it is a non-electrolyte[6]. Analytical data: Calculated for $Cu(TBTC)_2(CH_3$–

COO)$_2$: N, 7.80%; S, 8.9%; Cu, 8.80%; C, 63.20; H, 4.71. Found: N, 8.07%; S, 9.15%; Cu, 8.50%; C, 63.50; H, 4.75.

Cu(TBTC)$_2$ (CH$_3$COO)$_2$ is diamagnetic[7-9] in the solid state, indicating that it is either a dimeric/polymeric copper(II) complex or a copper(I) complex. The visible and ESR spectra (discussed before), however, show that it is a dimeric copper(II) complex.

The ligand shows bands at 3265 and 1670 cm^{-1} which are assigned to ν_{NH} and $\nu_{C=O}$ modes respectively. The first band remains unchanged while the second band shows a negative shift of 10 cm^{-1} in the complex, suggesting non-involvement of the nitrogen and involvement of carbonyl oxygen in coordination to the metal ion. The bands at 1530, 1275, 1150 and 820 cm^{-1} in the spectrum of the ligand are assigned[10-12] to thioamide bands I, II, III and IV respectively. The thioamide IV band is observed at 780 cm^{-1} in the complex, showing thereby that the sulphur atom is bonded in thione form in the complex. The coordination of sulphur to the metal ion is further supported by a positive shift of 5–15 cm^{-1} of the thioamide I, II and III bands. The two absorption bands appear at 1700 and 1400 cm^{-1} ($-\Delta\nu_{OCO} = 300$ cm^{-1}) in the complex, suggesting the presence of unidentate[13] carboxylate group in the adduct.

Cu(TBTC)$_2$(CH$_3$COO)$_2$ is one of the few copper(II) complexes[10,14,15] in which spin paramagnetism is completely quenched (μ_{eff} = zero) at room temperature. The diamagnetism of the complex may be attributed to the super-exchange phenomenon and/or highest polarizability[9,16] of the ligand (sulphur donor), which supplies more electron density to copper ions, and consequently the ions interact more strongly, resulting in a diamagnetic complex. The strong spin–spin interaction may take place by the overlap of singly occupied $d_{x^2-y^2}$ orbital on the two cupric ions. In the absence of detailed X-ray work, it is difficult to decide between these alternatives.

Harris *et al.*[7], however, explained diamagnetism of the copper(II) complex of diazoaminobenzene on the basis of metal–metal interaction and attributed two bands at 14925 and 18870 cm^{-1} in the visible spectrum of the complex to *d–d* transitions, assuming square planar geometry around copper(II). Agarwala *et al.*[15] observed three bands at 12990, 19610 and 24390 cm^{-1} in the electronic spectrum of a light yellow diamagnetic copper(II) complex with 2-mercaptobenzothiazole. The first two bands were attributed to *d–d* transitions in square planar geometry and the third one to the dimeric copper(II) species. The electronic spectrum of the yellow diamagnetic complex Cu(TBTC)$_2$(CH$_3$COO)$_2$ also yields three bands at 15390, 18520 and 23260 cm^{-1}. The first two bands may be assigned to *d–d* transitions in distorted octahedral environment around copper(II) ion and the third one to dimeric copper(II) species.

The X-band spectrum of powdered sample of Cu(TBTC)$_2$(CH$_3$COO)$_2$ shows a band at 1700 G ($g = 4.005$), which may be attributed to $\Delta M_S = 2$ transition arising from the presence of an intramolecular electron exchange between two copper(II) ions in the dimeric species[17]. The parallel and perpendicular features are broadened and not clearly resolved, probably owing to the badly resolved ligand hyperfine interaction.

The ligand (TBTC) and the complex Cu(TBTC)$_2$ (CH$_3$COO)$_2$ were tested for fungicidal activity against *Rhizoctonia solani* Kuhn at 50, 100 and 300 ppm. Both show no toxicity at 50 and 100 ppm but have 17 and 11% fungicidal activity at 300 ppm respectively.

Based on chemical composition and the physico-chemical properties discussed above an octahedral structure is tentatively proposed for the complex.

The author thanks Dr B. Singh and Prof. R. C. Aggarwal (former Head), Department of Chemistry, Banaras Hindu University, Varanasi, for helpful discussion and provision of facilities for micro-analyses, magnetic measurements and recording IR and electronic spectra. Thanks are also due to RSIC, IIT, Bombay, for recording ESR spectrum and to UGC, New Delhi, for financial assistance.

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HETEROCYCLIC SYSTEMS CONTAINING BRIDGEHEAD NITROGEN ATOM: SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF *p*-BIS(*s*-TRIAZOLO[3, 4-*b*] [1,3,4]THIADIAZIN-3-YL)PHENYLENE AND *p*-BIS(*s*-TRIAZOLO[3, 4-*b*] [1, 3, 4] THIADIAZOL-3-YL)PHENYLENE

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IN continuation of our earlier studies on the synthesis of condensed biologically active triazole systems[1-3], we report in this paper the synthesis of some heterocyclic systems derived from *p*-bis(5-mercapto-4-amino-*s*-triazol-3-yl)phenylene (I) in order to explore the possibility of the synthesis of heterocyclic systems with appreciable biological activity.

Compound I was prepared in excellent yield by the reaction of terephthalic acid bis-hydrazide with CS_2 and hydrazine following the method of Reid and Heindel[4].

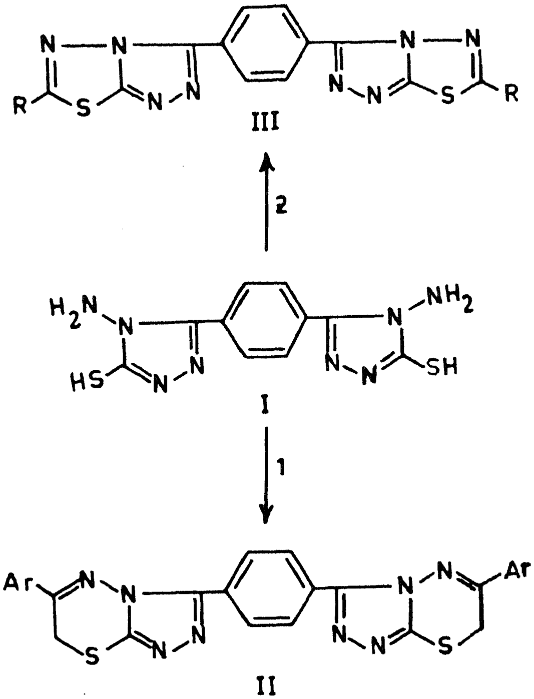
The reaction of I with α-haloketones and carboxylic acids in absolute ethanol yielded *p*-bis (7*H*-6-aryl-*s*-triazolo [3,4-*b*] [1,3,4]thiadiazin-3-yl) phenylene (II) and *p*-bis(6-aryl-*s*-triazolo[3,4-*b*] [1,3,4]thiadiazol-3-yl)phenylene (III) respectively in good yields.

The structures of II and III are compatible with their elemental analysis and spectroscopic data. The absence of C=O band in the IR spectra of compounds II and III corroborates the cyclic structures in these compounds.

Melting points reported are uncorrected. Thin-layer chromatography was performed on silica gel plates using acetone–benzene (2:3) as irrigant. IR spectra ($\nu_{max}cm^{-1}$) in nujol were recorded on a Beckman IR-20 spectrometer.

p-Bis(5-mercapto-4-amino-s-triazol-3-yl)phenylene(I)

It was prepared from terephthalic acid bis-hydrazide following the method of Reid and Heindel[4], m.p. 280°C (d) (EtOH), yield 52%. Found: N, 36.85; S, 20.91. $C_{10}H_{10}N_8S_2$ requires N, 36.60; S, 20.92%. IR; 820 (*p*-disubstituted benzene ring), 1500 (C–N stretching), 1600 (C=C and C=N), 2600 (S–H stretching), 3020 (C–H stretching, aromatic), 3220, 3400 (N–H stretching, NH_2 group).



1. $ArCOCH_2X$; 2. RCOOH

Scheme 1

p-Bis (7H-6-p-bromophenyl-s-triazolo [3,4-b] [1,3,4] thiadiazin-3-yl)phenylene (IIa, Ar = p-Br–C_6H_4–)

A mixture of I (1.53 g, 0.005 mol) and *p*-bromophenacyl bromide (2.78 g, 0.01 mol) in absolute ethanol (150 ml) was heated under reflux for 6 h. The salt separated was filtered and basified with ammonia solution. The solid thus obtained was filtered, washed with water and finally with hot ethyl alcohol; m.p. 225°C, yield 1.6 g (48.2%). Found: C, 47.23; H, 2.72; N, 16.43; S, 9.89. $C_{26}H_{16}N_8S_2Br_2$ requires C, 46.99; H, 2.41; N, 16.87; S, 9.64%. IR: 800 (1,4-disubstituted benzene ring), 1580, 1600 (C=C, C=N), 3060 (C–H stretching, aromatic).

The data for other *s*-triazolothiadiazines (II) prepared similarly are given in table 1.

p-Bis (6-p-chlorophenyl-s-triazolo [3,4-b] [1,3,4] thiadiazol-3-yl)phenylene (IIIa, R = p-Cl–C_6H_4–)

A mixture of I (1.53 g, 0.005 mol), *p*-chlorobenzoic acid (1.57 g, 0.01 mol) and $POCl_3$ (10 ml) was heated on a water bath for 1 h. The reaction mixture was cooled to room temperature and poured into ice-cold water. The resultant solid obtained was filtered, washed with water and crystallized from glacial acetic acid as yellow-coloured crystalline mass; m.p. 255°C, yield 1.7 g (62%). Found: C, 52.96; H, 2.46; N, 20.13; S, 11.35. $C_{24}H_{12}N_8S_2Cl_2$ requires C, 52.65; H, 2.19; N, 20.48; S, 11.70%. IR: 830 (1,4-disubstituted benzene), 1560, 1590 (C=C and C=N), 3050 (C–H stretching, aromatic).

The data for other *s*-triazolothiadiazoles (III) prepared similarly are given in table 2.

Antimicrobial activity

The compounds II (Ar = *p*-Br–C_6H_4–) and III (R = *p*-Cl–C_6H_4–) were tested for antimicrobial activity against *Staphylococcus aureus*, (a gram-positive bacterium), *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative bacteria) and *Candida albicans* (a fungus) by serial plate dilution method[5].

The minimum inhibitory concentration of compound III against *E. coli* and *S. aureus* was 125 μg/ml.

Compound II showed activity against *E. coli* and *S. aureus* when used undiluted and may be used for local application in the form of powder or ointment

Table 1 *Characterization data for s-triazolo thiadiazines (II)*

Ar	m.p. (°C)	Yield (%)	Mol. formula	Found (calc.) (%) C	H	N	S
p-Cl–C_6H_4–	198	52.2	$C_{26}H_{16}N_8S_2Cl_2$	54.62 (54.26)	2.41 (2.78)	19.86 (19.48)	11.53 (11.13)
p-NO_2–C_6H_4–	210(d)	61.6	$C_{26}H_{16}N_{10}S_2O_4$	52.75 (52.35)	2.28 (2.68)	— (—)	10.23 (10.74)
p-C_6H_5–C_6H_4–	176	63.9	$C_{38}H_{26}N_8S_2$	69.70 (69.30)	3.58 (3.95)	16.82 (17.02)	10.12 (9.73)

Table 2 *Characterization data for s-triazolo thiadiazoles (III)*

R	m.p. (°C)	Yield (%)	Mol. formula	Found (calc.) (%) C	H	N	S
C_6H_5–	200	56.5	$C_{24}H_{14}N_8S_2$	60.42 (60.25)	3.22 (2.93)	22.11 (22.43)	13.71 (13.39)
o-Cl–C_6H_4–	250	54.9	$C_{24}H_{12}N_8S_2Cl_2$	52.15 (52.65)	2.52 (2.19)	20.82 (20.48)	11.36 (11.70)
m-Cl–C_6H_4–	228	60.0	$C_{24}H_{12}N_8S_2Cl_2$	52.98 (52.65)	2.46 (2.19)	20.22 (20.48)	11.96 (11.70)
p-CH_3–C_6H_4–	220	53.0	$C_{26}H_{18}N_8S_2$	61.34 (61.66)	3.18 (3.56)	21.92 (22.12)	12.89 (12.65)
p-CH_3O–C_6H_4–	235	66.9	$C_{26}H_{18}N_8S_2O_2$	58.34 (57.99)	3.83 (3.35)	20.61 (20.82)	11.56 (11.90)
2,4-Cl_2–C_6H_3–	228	58.0	$C_{24}H_{10}N_8S_2Cl_4$	46.32 (46.75)	1.94 (1.62)	18.58 (18.18)	9.89 (10.39)

provided further studies indicate absence of toxicity following local application.

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SYNTHESIS AND CHARACTERIZATION OF SOME NEW 3-ACETYL-4-ARYL-2-PYRAZOLINES

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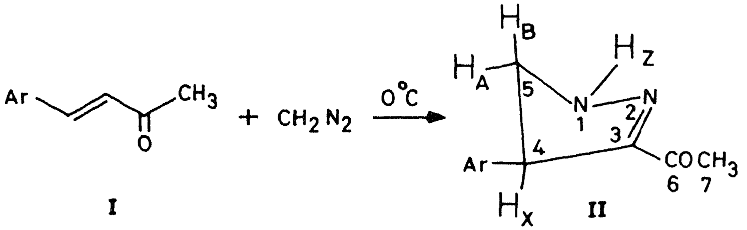
SYNTHESIS of pyrazolines through cycloaddition of diazomethane to activated olefines has been known for a long time[1]. However, there is no general agreement on the structure of 2-pyrazolines[2-6]. Recently, we have established the structure of 3-aroyl-4-aryl-2-pyrazolines[7]. In continuation of this study, we report in this communication the synthesis and characterization of 3-acetyl-4-aryl-2-pyrazolines (IIa–h).

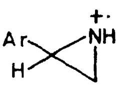
4-Aryl-3-buten-2-ones (Ia–h) were obtained by crossed aldol condensation of appropriately substituted aldehydes and acetone[8]. The ether solution of diazomethane prepared from nitrosomethylurea[9] was added to butenones (Ia–h) in ether at 0°C to give 3-acetyl-4-aryl-2-pyrazolines (IIa–h). In the case of Ia, the formation of 1-pyrazoline[1] was noticed, but this changed to 2-pyrazoline, IIa.

The IR data (table 1) for the pyrazolines (IIa–h) indicate bands in the regions 3280–3340, 1630–1650 and 1520–1530 cm^{-1} assigned to NH, C=O and C=N stretch vibrations respectively. It is significant to note that $\nu_{C=N}$ shifted to lower frequencies while $\nu_{C=O}$ shifted to higher frequencies compared to those of 3-aroyl-4-aryl-2-pyrazolines[5-7].

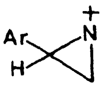
^{1}H NMR spectroscopic data (table 2) contain ABX pattern of signals attributed to 4-methine and 5-methylene protons. It is interesting to note that 200 MHz spectra clearly resolved the NH signal, whereas in 90 MHz spectra the signal could not be traced out. In case of IIb and IIc the coupling with NH proton is also discernible.



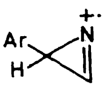
Ar		Ar	
(a)	Phenyl	(e)	4-Chlorophenyl
(b)	4-Methylphenyl	(f)	3,4-Methylenedioxyphenyl
(c)	4-N, N-dimethylphenyl	(g)	4-Methoxyphenyl
(d)	2-Chlorophenyl	(h)	Furan-2-yl



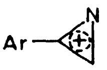
m$_1$



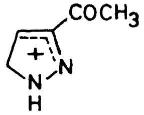
m$_2$



m$_3$



m$_4$



m$_5$

Table 1 *Physical and chemical data for 2-pyrazolines (IIa–h)*

Compound	Reaction time (h)	Yield* (%)	m.p. (°C)	Mol. formula	Found (Calc.) (%)			UV (MeOH) (nm)	IR** (cm^{-1})		
					C	H	N		C=N	C=O	N–H
IIa	60	85	102–103[a]	$C_{11}H_{12}N_2O$	70.58 (70.21)	6.57 (6.30)	14.79 (14.89)	309	1530	1640	3280
IIb	48	92	81–83	$C_{12}H_{14}N_2O$	70.55 (71.29)	7.16 (6.93)	13.05 (13.86)	311	1530	1635	3340
IIc	48	66	124–125	$C_{13}H_{17}N_3O$	66.47 (67.53)	7.62 (7.35)	17.71 (18.18)	308 255	1520	1635	3290
IId	36	83	120–121	$C_{11}H_{11}N_2OCl$	59.62 (59.19)	4.35 (4.93)	12.49 (12.55)	318 304	1530	1630	3280
IIe	24	90	97–99	$C_{11}H_{11}N_2OCl$	59.07 (59.19)	4.64 (4.93)	11.70 (12.55)	308	1520	1630	3310
IIf	24	86	94–95	$C_{12}H_{12}N_2O_3$	62.20 (62.06)	5.75 (5.17)	11.88 (12.07)	307 296	—	1650	3320
IIg	36	62	90–91	$C_{12}H_{12}N_2O_2$	65.84 (66.67)	6.83 (5.56)	12.69 (12.96)	309	1530	1630	3340
IIh	36	75	53–54	$C_9H_{10}N_2O_2$	61.39 (60.67)	5.64 (5.62)	15.43 (15.73)	305	—	1650	3340

*Computed based on isolated compounds. **Samples as KBr pellets (II a, b, c, d, e)/$CHCl_3$ solutions (II f, g, h). [a]Lit.[1] m.p. 100.5–101°C.

Table 2 *^1H NMR spectroscopic data* for pyrazolines (IIa–h)*

Compound	H_A	H_B	H_X	H_Z	J_{AB}	J_{AX}	J_{BX}	J_{BZ}	7-CH_3	Ar-H	Substituents
IIa	3.71	4.03	4.39	6.25	11	5	11	—	2.38	7.19–7.32 (5H, *m*)	—
IIb	3.68	4.03	4.36	6.22	11	5	11	2	2.38	7.05–7.15 (4H, *m*)	2.30 (3H, *s*)
IIc	3.67	3.98	4.31	6.18	11	5	11	2	2.37	6.65 (2H, *d*, *J*=9Hz) 7.09 (2H, *d*, *J*=9Hz)	2.90 (6H, *s*)
IId	3.54	4.05	4.81	6.38	11	6	11	—	2.45	6.38–7.39 (4H, *m*)	—
IIe	3.68	4.05	4.38	—	11	5	11	—	2.40	7.10–7.35 (4H, *m*)	—
IIf	3.65	4.01	4.35	—	11	5	11	—	2.42	6.75 (3H, *s*)	5.95 (2H, *s*)
IIg	3.65	4.02	4.35	—	11	5	11	—	2.40	6.82 (2H, *d*, *J*=9Hz) 7.20 (2H, *d*, *J*=9Hz)	3.80 (3H, *s*)
IIh	3.88	3.88	4.56	—	—	8	10	—	2.46	6.10 (1H, *m*) 6.30 (1H, *m*) 7.35 (1H, *m*)	—

*Solvent, $CDCl_3$; δ values from TMS; 200 MHz (IIa, b, c, d)/90 MHz (IIe, f, g, h) spectra.

Table 3 *^{13}C NMR spectroscopic data* for pyrazolines (IIa–h)*

Compound	C-3	C-4	C-5	C-6	C-7	Aromatic carbons						Substituents
						C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	
IIa	152.5	47.0	58.1	193.3	25.7	141.0	127.0	128.5	127.0	128.5	127.0	—
IIb	152.9	46.8	58.2	193.3	25.7	136.5	126.9	129.3	138.0	129.3	126.9	20.9
IIc	153.5	46.4	58.1	193.4	25.8	128.8	127.7	112.8	149.6	112.8	127.7	40.5
IId	151.2	44.2	57.3	193.2	25.7	137.9	133.1	129.7	128.3	127.1	127.7	—
IIe	151.9	46.3	57.8	193.2	25.5	139.6	128.4	128.6	132.5	128.6	128.4	—
IIg	152.0	45.8	57.8	193.2	25.3	133.2	127.7	113.6	158.1	113.6	127.7	54.7
IIh	152.4	40.0	55.0	193.1	25.2	—	148.2	105.5	110.1	141.2	—	—

*Solvent; $CDCl_3$; δ values from TMS; 50.16 MHz (IIa, b, c, d)/22.63 MHz (IIe, g, h) spectra; Assignments are supported by SFORD data.

Table 4 *Important mass spectral fragments of IIa–h m/z (%)*

Compound	M^+	M^+–H	M^+–CH_3	M^+–$COCH_3$	m_1	m_2	m_3	m_4	m_5	$ArCHCH_2^+$	$ArCHCH^+$	Other prominent fragments
IIa	188 (100)	187 (15.2)	173 (8.1)	145 (53.5)	119 (14.1)	118 (39.0)	117 (64.6)	116 (42.4)	111 (14.1)	104 (23.2)	103 (10.1)	91(43.4); 90(31.3); 89(42.5); 77(33.3)
IIb	202 (100)	201 (19.2)	187 (5.1)	159 (56.0)	133 (18.2)	132 (53.0)	131 (82.0)	130 (36.4)	111 (12.2)	118 (31.3)	117 (37.4)	105(40.4); 103(29.3); 91(38.4); 77(20.2)
IIc	231 (100)	230 (5.2)	—	188 (1.1)	162 (3.1)	161 (29.4)	160 (33.3)	159 (35.3)	—	147 (5.9)	146 (5.1)	133(7.2); 43(3.1)
IId	222 (5.5) 224 (5.1)	221 (3.4) 223 (5.8)	207 (3.4) 209 (3.1)	179 (12.8) 181 (4.1)	153 (9.6)	152 (7.8)	151 (13.1)	150 (11.2)	111 (4.1)	138 (5.1)	—	187(100); 125(8.1); 43(97.0)
IIe	222 (100)	221 (29.3)	207 (8.0)	179 (70.7)	153 (50.0)	152 (50.5)	151 (57.4)	150 (51.5)	111 (14.2)	138 (8.1)	137 (33.3)	116(26.3); 115(30.3); 114(41.4); 110(28.3); 89(69.7)
	224 (33.4)	223 (21.2)	—	181 (31.3)	155 (7.0)	154 (10.1)	153 (50.0)	152 (50.5)		140 (10.1)		
IIf	232 (68.9)	231 (7.2)	—	189 (9.3)	163 (9.8)	162 (33.4)	161 (46.5)	160 (20.2)	111 (3.1)	148 (19.2)	147 (17.2)	135(22.2); 43(100)
IIg	218 (93.9)	217 (11.2)	203 (1.1)	175 (21.4)	149 (12.3)	148 (65.7)	147 (53.1)	146 (28.6)	111 (4.6)	134 (25.0)	133 (10.1)	132(26.9); 121(32.7); 91(17.9); 43(100)
IIh	178 (36.6)	177 (4.2)	163 (5.9)	135 (9.7)	109 (6.2)	108 (32.7)	107 (64.3)	106 (19.4)	—	94 (27.6)	93 (3.1)	43(100)

^{13}C NMR data (table 3) show signals in the regions 40–47 ppm (doublets in SFORD) and 55–58 ppm (triplets) which are interpreted[7] as due to C-4 and C-5 respectively. The chemical shifts of the aromatic carbons show the substituent shifts as expected[10].

The mass spectral fragments (table 4) agree well with the structures assigned. Some of the fragmentation pathways are similar to those for 3-aroyl-4-aryl-2-pyrazolines[5].

From the above it is clear that 3-acetyl-4-aryl-2-pyrazolines have the structure II. Such a structure assignment has also been favoured by Smith *et al.*[1], but without any spectroscopic evidence.

3-Acetyl-4-aryl-2-pyrazolines: General procedure

To a solution of Ia (1.46 g, 10 mmol) in ether or chloroform at 0°C was added excess of diazomethane (from 4 g of nitrosomethylurea) and the mixture kept at 0°C for 60 h. The progress of the reaction was monitored over silica gel layers. After completion of the reaction, the solvent was removed under vacuum. The product IIa, 1.6 g, formed was recrystallized from pet. ether–ether; m.p. 102–103°C.

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15 December 1988

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TWO NANNANDROUS SPECIES OF *OEDOGONIUM* LINK, NEW TO INDIAN ALGAL FLORA

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THE genus *Oedogonium* Link, is world-wide in distribution and most of the species are found epiphytic on *Cladophora*, *Pithophora*, *Chara* and *Nitella*, and also on larger species of *Oedogonium* and *Bulbochaete*[1]. At present the genus includes more than 531 species[1,2] excluding its varieties and forma. Of these, 171 nannandrous species, completely described, and 10 species, incompletely described, are included in a monograph by Gonzalves[1]. Among them 58 nannandrous species with their 73 taxa are so far reported from India.

During fortnightly collections of epiphytic algae of Mauri Lake, Pratapgarh, UP, from August 1984 to July 1986, 65 taxa of the genus *Oedogonium* were encountered. Among them 18 taxa are nannandrous, of which only two taxa, viz. *Oedogonium sinuatum* (Trans.) Tiffany f. *seriatum* Prescott and *O. westii* (Tiffany *et* Braun) Tiffany, are described here. Both species are new additions to the Indian algal flora.

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†For correspondence

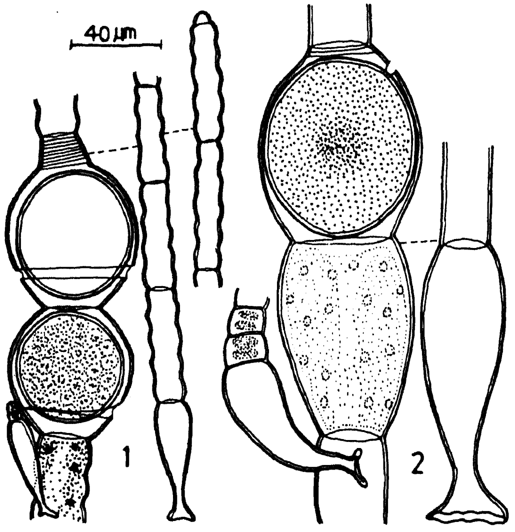
Oedogonium sinuatum (Trans.) Tiffany f. *seriatum* Prescott (figure 1).

Filaments nannandrous, gynandrosporous; vegetative cells undulate and capitellate, undulation up to four, 20–30 μm in diameter, 50–90 μm in length; oogonia single or up to four, seriate, spherical, 60–70 μm in diameter, 50–80 μm in length, operculate, division inferior; oospores spherical, smooth-walled, not completely filling the lumen of the oogonia, 55–60 μm in diameter, 50–60 μm in length; nannandria usually many, up to six, attached with a suffultory cell, 8–10 μm in diameter, 40–50 μm in length; antheridia exterior, two, 8–9 μm in diameter, 9–12 μm in length.

The present specimen agrees well with the type description. However, *O. undulatum* (Wittrock) Hirn and *O. sinuatum* (Trans.) Tiffany are quite similar in general appearance, but both have narrower vegetative cells and oogonia in comparison to the present taxon.

Habitat and collection no.: Epiphytic on *Ceratophyllum demersum*, *Utricularia aurea*, *Nelumbo nucifera* and *Chara zeylanica*; 47, 113.

Occurrence and distribution: August to March; this alga is new for Indian algal flora; earlier reported from N. America[1].



Figures 1 and 2, **1,** *Oedogonium sinuatum* (Trans.) Tiffany f. *seriatum* Prescott. **2,** *Oedogonium westii* (Tiffany *et* Braun) Tiffany.

Oedogonium westii (Tiffany *et* Braun) Tiffany (figure 2).

Filaments nannandrous, gynandrosporus; vegetative cells cylindrical, 15–30 μm in diameter, 70–140 μm in length, suffultory cell inflated, 35–55 μm in diameter, 70–87 μm in length; oogonia two or up to three, seriate, obovoid, 50–64 μm in diameter, 65–85 μm in length, poriferous, pore superior; oospores ovoid to obovoid, smooth-walled and filling the lumen of oogonia, 52–61 μm diameter, 60–75 μm in length; nannandria curved and situated on a suffultory cell, 17–25 μm in diameter, 65–75 μm in length; antheridia single, 15–19 μm in diameter, 10–12 μm in length.

The present specimen resembles the type description in all the essential features.

Habitat and collection no.: Epiphytic on *Ceratophyllum demersum*, *Hydrilla verticillata* and *Eleocharis dulcis*; 123, 191.

Occurrence and distribution: August to December; the present alga is also new to Indian algal taxonomy; previously it was reported from N. America and Europe[1].

25 August 1988; Revised 4 February 1989

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LEAF ANATOMY OF MIDRIBLESS MUTANTS IN PEARL MILLET

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THREE spontaneous midribless mutants in pearl millet [*Pennisetum glaucum* (L.) R. Br., syn. *P. typhoides* (Burm.) S. & H.], two from Mali (IP 6534 and IP 10154) and one from India (J 561), were identified. All the leaves of the midribless mutants characteristically droop in appearance (figure 1) and

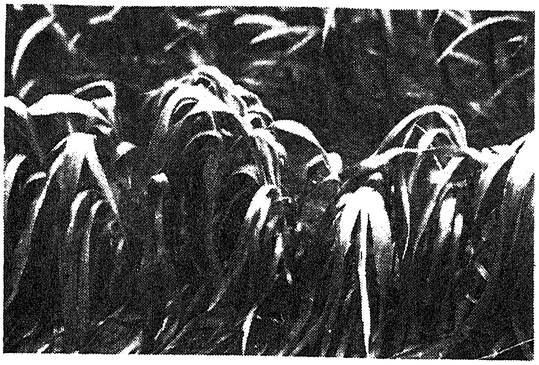
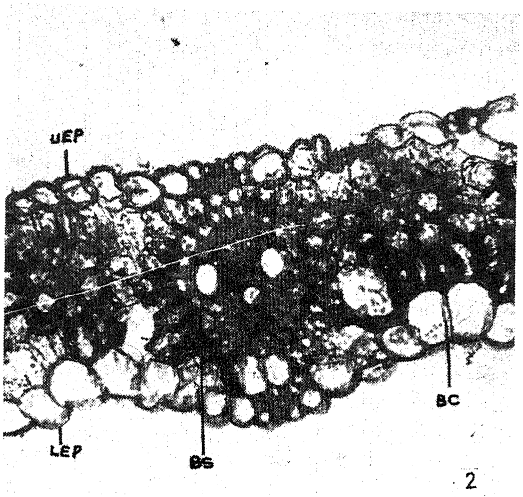


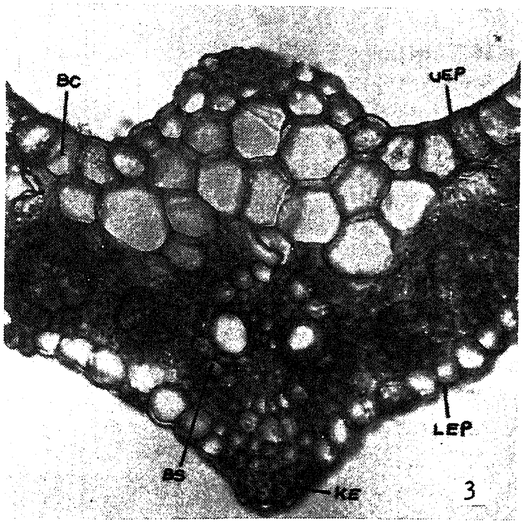
Figure 1. Morphology of the midribless mutant (IP 6534) of pearl millet, showing drooping leaves.

are distinguishable from emergence to maturity[1]. The normal leaf blade has a deep groove on the abaxial side and is curved inwards, while on the adaxial side there is a prominent midrib; in the mutants, both abaxial and adaxial sides are alike, the leaf blades are flat and ribbon-like, and the veins are not so prominent. The anatomical differences observed between the leaves of normal and midribless mutants are reported in this note.

Fresh leaf material of 1-month-old plants was collected and fixed in FPA (40% formalin, propionic acid, and 70% ethyl alcohol, in 5:5:90 proportion by volume). The methods used by Maiti and Bisen[2] for making leaf sections and staining were followed.

In both the normal and the midribless mutants, the epidermis is cuticularized and composed of rectangular to oval-shaped cells. The lower epidermis is entire in outline, but the upper epidermis is wavy and interspersed with zones of bulliform (motar) cells. The midrib in transverse section appears 'semi-lunar'. The main difference observed is that a prominent keel with parenchymatous tissue was present on the abaxial side in the normal leaves but was absent in the mutant (figures 2 and 3). The number of vascular bundles in the leaf was also different in the normal and the mutant plants. In the normal leaves, 5–9 large and 20–30 small vascular bundles were observed, while only one large and 10–15 small vascular bundles were observed in the midribless mutants. The amount of sclerenchymatous tissue associated with large bundles was reduced in the mutant compared to that in the normal. Though two non-allelic genes *mrl*$_1$ or *mrl*$_2$ control the midribless trait[1], differences in leaf anatomy were not found among them. Vascular bundles in both the cases were seen surrounded by two parenchymatous sheaths; this is not a common feature in mesophytic grasses[3]. The cells of the outer sheath are very large in size compared to those of the inner sheath. Mesophyll cells surrounding the vascular bundles are radially arranged.





Figures 2 and 3. **2,** Transverse section of midribless leaf, showing absence of a prominent keel. **3,** Transverse section of normal leaf, showing prominent keel on the abaxial side. (UEP, Upper epidermis; LEP, lower epidermis; BS, bundle sheath cells; BC, bulliform cells; KE, keel.)

'Kran z' anatomy, i.e. presence of chloroplasts in the mesophyll as well as in bundle sheath cells, which is a characteristic feature of C4 plants, was observed in both normal and mutant plants[2]. The absence of a prominent keel and reduced sclerenchymatous tissue on both abaxial and adaxial sides of the large vascular bundle in the midribless mutant cause the leaf to droop.

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NEW REPORT OF WILT DISEASE OF BRINJAL IN INDIA

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DURING the regular disease survey at Mahatma Phule Agricultural University, Rahuri, and Ganeshkhind garden, Pune, an incidence of *Fusarium* wilt was noticed on brinjal (*Solanum melongena* L.) varieties Manjari Gota and Vaishali during 1984–85. The characteristic initial disease symptom under field conditions was sudden wilting of leaves of infected plants. Thereafter, loss of green colour of the foliage and, finally, browning were seen. The disease developed by infection of xylem vessels. The affected leaves showed vein clearing of young leaflets and epinasty of old leaves, followed by general yellowing and defoliation. The wilting started in the lower parts of the plants and spread upwards and was generally observed at flowering stage. Sometimes partial wilting was also observed (figure 1), and the plants wilted completely within 10–12 days. The affected plants showed stunted growth, and when the infected roots were split open, browning of vascular tissues was observed.

Steeketenbug[1] first reported *Fusarium* wilt disease of brinjal caused by *Fusarium oxysporum* f. sp. *melongenae* from the Netherlands. However, Laxminarayana and Reddy[2] reported post-harvest disease of brinjal fruits caused by *F. oxysporum* Schl. from Warangal, India.

Potato, tomato, pigeonpea, chickpea and coriander also appeared to be hosts of this organism[3].

In the present investigation, the causative organism was isolated in pure culture and was identified as *Fusarium oxysporum* f. sp. *melongenae*. The pathogenicity of the fungus was proved by soil inoculation method. Typical wilt symptoms were observed 60 days after transplanting (figure 2), i.e. at flowering stage, and reisolated culture was used for carrying out further studies.

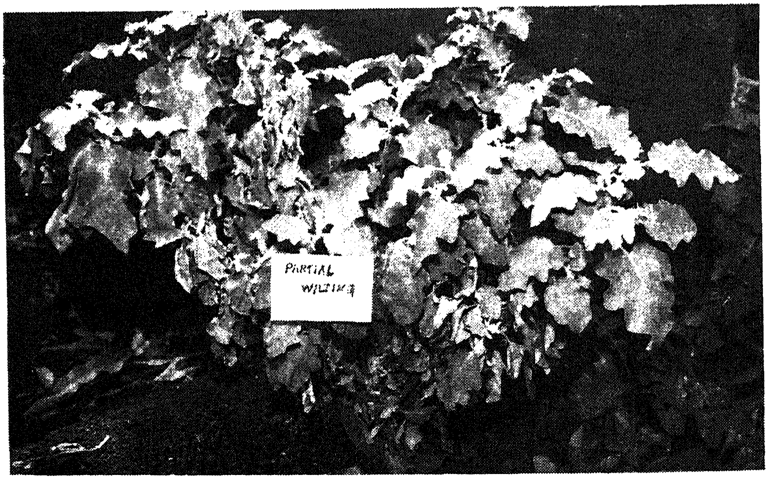

Figure 1. Brinjal, showing partial wilting caused by *Fusarium*.

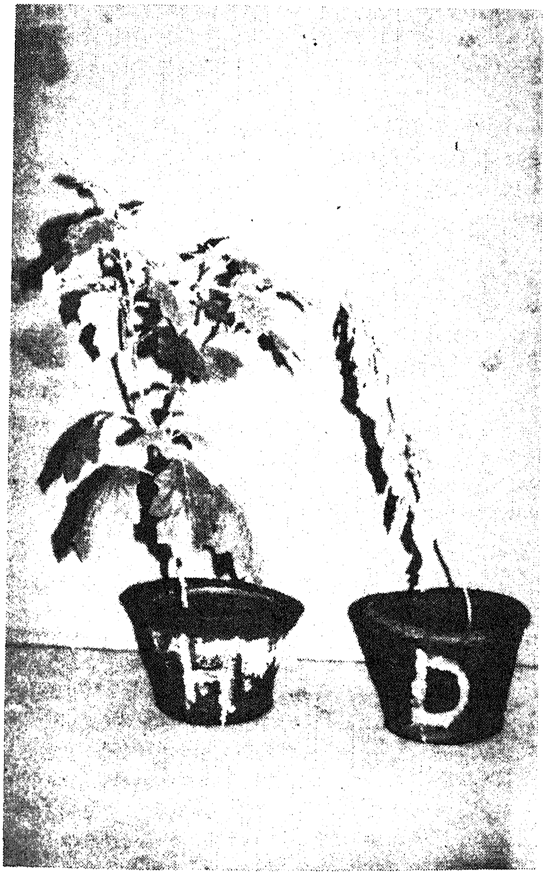

Figure 2. Demonstration of pathogenicity of fungus isolated from brinjal affected by *Fusarium* wilt.

The culture of the fungus has been deposited in our Department's culture collection.

The authors thank Dr V. G. Rao, Mycologist, Maharashtra Association for the cultivation of Science, Pune, for identification of the fungus.

10 November 1988; Revised 11 April 1989

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PEROXIDASE AND POLYPHENOL OXIDASE ACTIVITIES IN SORGHUM AND *PERONOSCLEROSPORA SORGHI* INTERACTION

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PERONOSCLEROSPORA SORGHI (Weston & Uppal) C. G. Shaw, causes sorghum downy mildew (SDM), a major problem in sorghum [*Sorghum bicolor* (L.) Moench] production[1-3]. Use of disease-resistant varieties is a promising method of disease control. Bhat *et al.*[4] reported hypersensitive reaction of resistant sorghum lines to *P. sorghi*. In many plant diseases, disease resistance is correlated with changes in certain oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO)[5-10]. The present study was undertaken to investigate changes in the activities of these enzymes in sorghum and *P. sorghi* interaction.

Sorghum lines DMS-652 (susceptible), and DMRS-I and QL-3 (resistant) were used. Seedlings were raised in 15 cm earthen pots (10 seedlings/pot) in a glasshouse. Ten-day-old seedlings were inoculated with *P. sorghi* by spraying a 5 ml conidial suspension (80,000 conidia/ml) per pot. The inoculated seedlings were covered with polythene bags. Healthy and inoculated leaves were collected separately for enzyme assay at 15, 30 and 60 h after inoculation.

One gram of sorghum leaf tissue was ground into a fine paste with 1 g of acid-washed sand in a mortar at 4°C. Five ml of cold 0.1 M sodium phosphate buffer of pH 6.5 was used for extraction. The extract was centrifuged at 6,000 *g* for 15 min at 4°C and the supernatant was used as enzyme source.

PO and PPO activities were determined by the method of Malik and Singh[11]. Protein content of the buffer extracts was determined according to Lowry *et al.*[12]

PO activity in healthy leaves of all the three sorghum lines tested was very low, ranging from 12 to 26 units/mg of protein, and did not alter significantly up to 60 h after inoculation (figure 1). Further, there is no difference in enzyme activity between resistant and susceptible lines. In contrast,

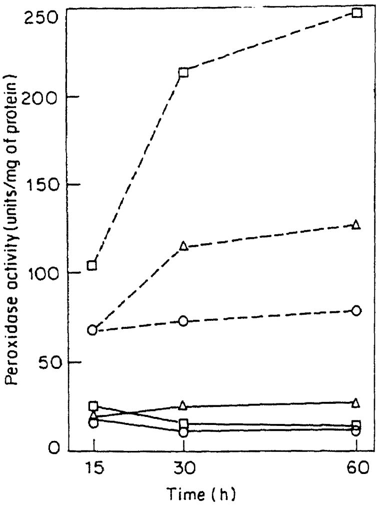

Figure 1. Peroxidase activity in healthy (solid lines) sorghum leaves and leaves inoculated with *Peronosclerospora sorghi* conidia (broken lines). (○, DMS-652; △, DMRS-I; □, QL-3.)

healthy plants of tomato resistant to *Verticillium dahliae*[13] and melons resistant to *Sphaerotheca fuliginea*[14] were reported to possess high PO activity.

Inoculation of *P. sorghi* increased PO activity in all the three sorghum lines, but the extent of increase varied among them. The highest increase in PO activity was noticed in QL-3.

PPO activity showed fluctuations similar to diurnal variation in healthy plants (figure 2A). However, in inoculated seedlings, PPO activity gradually increased over a period of time, without any fluctuation (figure 2B). The greatest increase was found in QL-3.

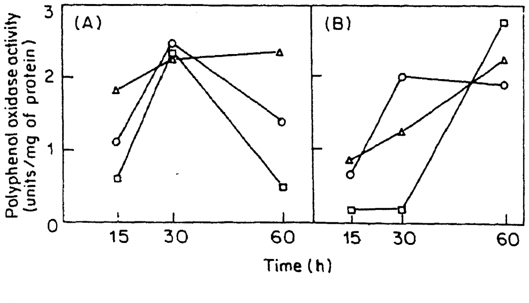

Figure 2. Polyphenol oxidase activity in **(A)** healthy sorghum leaves and **(B)** leaves inoculated with *Peronosclerospora sorghi* conidia. (○, DMS-652; △, DMRS-I; □, QL-3.)

Penetration by conidial germ-tubes of *P. sorg* takes place through stomata within 3–4 h inoculation[15]. Morphological differences during th process of infection were reported in leaves susceptible and resistant sorghum cultivars only 48 after inoculation with *P. sorghi*[16]. Our studi revealed changes in the concentration of oxidati enzymes as early as 15 h after inoculation wi *P. sorghi*. The continued increase in the activity oxidative enzymes correlates with the disea resistance of QL-3 and DMRS-I.

We thank ICRISAT, Hyderabad, for supplyin seeds of DMS-652 and QL-3; and Dr K. H. Anahosu Dharwar, for supplying seeds of DMRS-I. O thanks are also due to Dr R. G. Lalitha, Mr Mahes Joshi and Mr K. Gopal Marathe for help in enzym assays. One of the authors (PSBG) acknowledg financial assistance from CSIR, New Delhi.

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RECORD OF THE GENUS *KRUGERIA* (TENUIPALPIDAE, ACARI) FROM INDIA

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BAKER and Tuttle[1] erected the genus *Obuloides* to accommodate a unique tenuipalpid mite with one-segment palp and with seven pairs of dorsal setae, two of which were dorso-centrals and the other five marginal (humeral and dorso-lateral). This monotypic genus was from Coimbatore, India, on hibiscus. However, Meyer[2] erected *Krugeria*, which, like *Obuloides*, is characterized by one-segment palps and seven dorsal setae. The setae, however, unlike in *Obuloides*, are set on tubercles and arranged as follows: three pairs of dorso-centrals and four pairs of marginals. Further, members of *Krugeria* lack the transverse suture between dorso-central setae.

Recently, we collected two females and three nymphs of *Krugeria ramosa* Meyer on *Grewia orbiculata* Rottber (Tiliaceae) near Kalyani Dam, Tirupathi, South India. This is the first record of the genus from India. *K. ramosa* has been reported by Meyer[2] on *Grewia bicolor* and *G. monticola* from South Africa. Our record on *G. orbiculata* indicates that this mite is restricted to *Grewia*, which is found only in tropical Africa, Asia and Australian regions.

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SOME HISTOCHEMICAL STUDIES ON THE NEUROSECRETORY CELLS IN THE EYESTALK OF THE CRAB *POTAMON MAGNUM MAGNUM* (PRETZMAN)

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SEVERAL morphological and histochemical studies[1-3] on the neurosecretory cells in the cerebral and thoracic ganglia of the crab *Potamon magnum magnum* have been carried out. However, the histochemical nature of neurosecretory cells in the eyestalk of this decapod crustacean has not been studied earlier.

Adult *P. magnum magnum* were obtained from the Nawaran Spring, Iraq. Extirpated eyestalks were fixed in Bouin's, Carnoy's, alcoholic leadnitrate and Elftman's fixatives. They were then dehydrated in an alcohol series, cleared in xylol and terpenol, and embedded in histowax. Sections of 8 μm were cut and stained histochemically[4,5] for carbohydrates, proteins and lipids in the neurosecretory material.

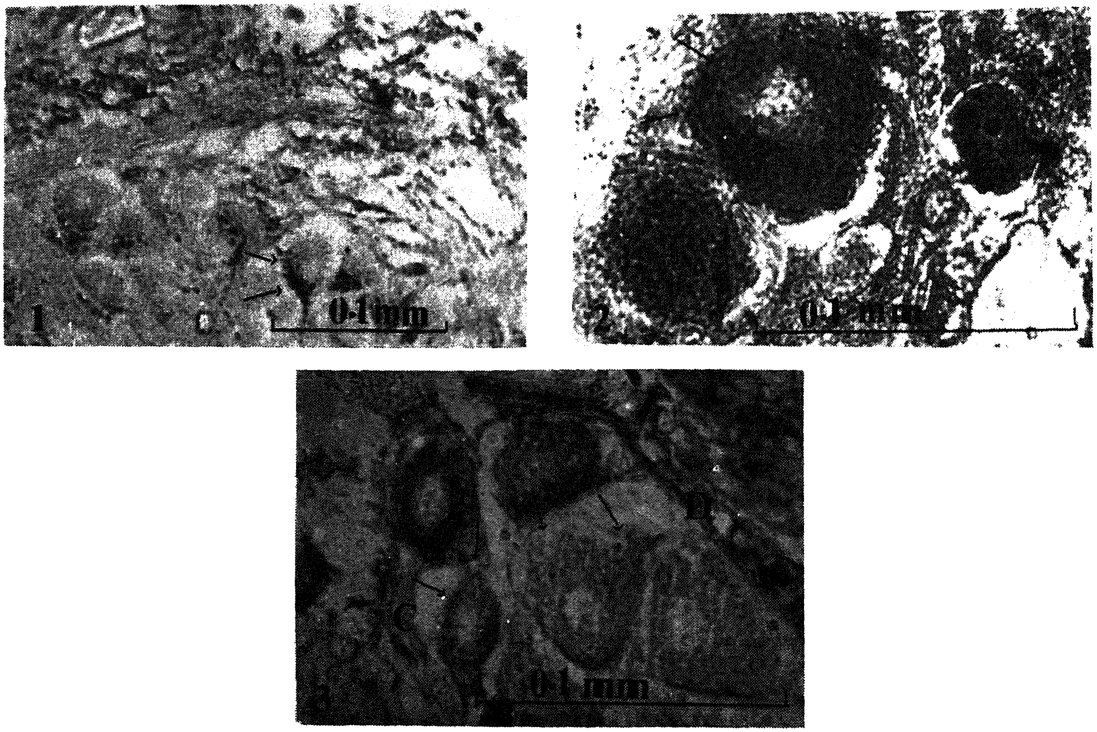
The results (table 1) indicate the presence of protein, carbohydrate and lipid in the neurosecretory material of the eyestalk of *P. magnum magnum*. Proteinaceous neurosecretory material has been detected[1-3] in the thoracic and cerebral ganglia of *P. magnum magnum*. Disulphide group-containing material has been reported[6] in neurosecretory material of axon endings in the sinus gland of *Carcinus maenas*. Proteinaceous neurosecretory material has been observed in the neurosecretory cells of *Chirocephalus diaphanus*[7,8] and *Paragrapsus gaimardii* and *Rivulogammarus syriacus*[9].

Neurosecretory material rich in proteins with SS and SH groups has been also detected[10] in the neurosecretory cells of the brain of *Artemia salina*. A hyperglycaemic hormone has been detected[11] in the neurosecretory system of the eyestalk of *Astacus leptodactylus*. Proteinaceous neurosecretory material

Table 1 *Histochemical reactions in the neurosecretory cells (A, B, C, D types) of the eyestalk of Potamon magnum magnum (Pretzman)*

	ME		MI			MT				
Technique	A	B	A	B	C	A	B	C	D	Specificity
Acrolein–Schiff (Van Duijn, 1961)	±	±	±	±	+	±	±	+	+	Proteins with NH, NH_2, SH and imidazole groups
Ninhydrin–Schiff (Yasuma and Itchikawa, 1953)	±	±	±	±	+	±	±	±	+	Protein-bound NH_2 groups
Millon reaction (Bensley and Gersh, 1933)	–	–	–	–	–	–	–	–	–	Tyrosine-containing proteins
Alkaline and tetrazolium (cited, Pearse, 1968)	±	±	±	±	±	±	±	±	±	Sulphydryl and disulphide groups
Periodic acid–Schiff (Hotchkiss, 1948)	±	±	±	±	+	±	±	+	++	Hexose-containing mucopolysaccharides
Toluidine blue (Kramar and Winder, 1953)	–	–	–	–	–	–	–	–	–	Metachromatic substances
Alcian blue (Steedman, 1950)	+	+	+	+	+	+	+	+	+	Acid mucopolysaccharides
Sudan black B (McManus, 1946)	±	±	±	±	+	±	±	+	+	Lipoproteins
Acetone Sudan black B (Berenbaum, 1954)	+	+	+	+	++	+	+	++	++	Masked lipids

ME, Medulla externa; MI, medulla interna; MT, medulla terminals. –, negative; ±, weakly positive; +, moderately positive; ++, strongly positive.



Figures 1–3. Horizontal sections through the eyestalk of *Potamon magnum magnum.* **1,** Arrows indicate C-type cells. PAS Alcoholic leadnitrate (× 180). **2,** Arrows indicate SBB-positive material in D cells. Sudan black B. Elftman's (× 560). **3,** Arrows indicate C and D cells. Acetone Sudan Black B. Elftman's (× 400).

has also been reported in the brain and optic ganglia of *Caridina fossarum*.

Periodic acid–Schiff (PAS) positive neuro-secretory material has been reported by many workers[1,3,6,7,11,12]. A, B and C type neurosecretory cells of the optic lobe of *P. magnum magnum* show weak to moderate positivity to PAS; type D cells show significant positivity. Similar results have been reported in *C. fossarum*[13].

Different results have been obtained for lipid components of neurosecretory material in Arthropoda. Earlier workers have found little or no lipid in the neurosecretory cells of different arthropods. However, a little to moderate amounts of lipids are detected in the neurosecretory cells of the eyestalk of *P. magnum magnum*.

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LACTATE DEHYDROGENASE IN THE GRASS CARP *CTENOPHARYNGODON IDELLA* (PISCES)

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Ctenopharyngodon idella, commonly known as grass carp, an exotic fish introduced into Indian aquaculture in 1959 to control submerged vegetation in freshwater bodies, has since become an important constituent in polyculture practices[1].

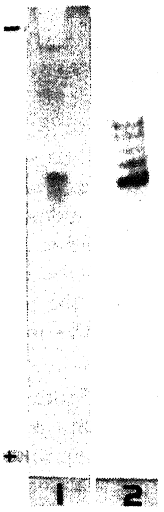
Lactate dehydrogenase (LDH, L-lactate: NAD oxidoreductase, E.C. 1.1.1.27), a polymorphic, multilocus enzyme, is a useful system in the understanding of differential gene expression in developmental stages and in different tissues of adults[2,3]. In most vertebrates, LDH has a tetrameric structure, and usually two different codominant loci have been identified which code for two subunits, A and B. These two subunits randomly associate with each other to form five tetrameric isozymes, viz. A_4, A_3B_1, A_2B_2, A_1B_3 and B_4. However, in many teleosts, there occurs a third locus, C, which may be expressed as a highly anodal band in eye and brain (eye-specific)[4-6] or as a slightly anodal (cathodal) band in liver (liver-specific)[6-8]. So far as the authors are aware, the expression of LDH isozymes in *C. idella* has not been studied earlier, either in developing stages or in adult tissues.

Healthy female and male brood fishes of *C. idella* were hormonally induced to release gametes in a specially designed 'breeding pool' in the Ganga Matsya Utpadan Kendra, Rathtala, West Bengal. The fertilized eggs were immediately collected and removed to a 'glass-jar hatchery' or to a 'Chinese hatchery' for smooth progress of development. The developing embryos were collected at suitable times and examined under the microscope to ascertain the developmental stage by identifying characteristic features (designated by serial numbers in table 1). The embryos were thoroughly washed in double-distilled water and then homogenized. The homogenates were centrifuged at 11,000 *g* for 30 min at 4°C. A known amount of the supernatant was used for electrophoresis. Polyacrylamide slab gel was prepared by following the methodology of Ornstein[9] and Davis[10]. Electrophoresis was carried out at 200 V and 3 mA/slot and the gel was then stained for LDH bands following the procedure of Nakano and Whiteley[11]. The relative mobility values of the bands were then calculated.

A single LDH band (figure 1) was observed in nine pre-hatching stages. But just before hatching, 5 electrophoretic bands appeared (figure 2) and persisted for 72 h after hatching. In the adult fish also, these five LDH bands were observed in muscle, heart, liver, kidney, eye and brain (figure 3). In addition, there was a lowly anodal band in the liver of adult. The staining properties and other details of the bands are listed in table 2.

Table 1 *Arbitrary designation of different developmental stages of Ctenopharyngodon idella*

Arbitrary stage	Hours (h) after fertilization (f)/ hatching (H)	Morphology
G-1	$\frac{1}{2}$ h f	Non-adhesive and non-floating zygote, cleavage started
G-2	$\frac{3}{4}$ h f	Cleavage continued
G-3	1 h f	Mass of cell over the yolk
G-4	2 h f	Blastodisc appeared
G-5	3 h f	Embryo enlarged in size and covered two thirds of the yolk surface
G-6	5 h f	– do –
G-7	7 h f	Eye-bud initiation
G-8	9 h f	The embryo with yolk-sac bean-shaped, head and tail distinguishable
G-9	11 h f	Twisting movement of the embryo inside the egg case
G-10	13 h f	Hatching
G-11	3 h H	Vitelline circulation started
G-12	6 h H	– do –
G-13	12 h H	No pigmentation over eyes
G-14	18 h H	Pigmentation over eyes
G-15	24 h H	Pigmentation over the eyes dense
G-16	36 h H	Dorsal fin developed
G-17	48 h H	– do –
G-18	60 h H	Yolk-sac reduced, gills appeared
G-19	72 h H	Complete dissolution of yolk-sac, free swimming fries



Figures 1 and 2. 1, LDH zymograms of pre-hatching and **2,** post-hatching stages showing 1 and 5 bands respectively.

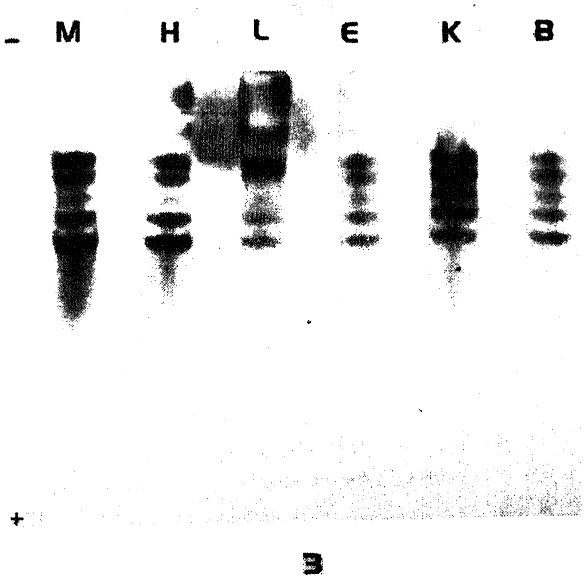


Figure 3. LDH zymograms of different tissues in adult *C. idella.* (M, muscle; H, heart; L, liver; E, eye; K, kidney; B, brain.)

Table 2 [illegible]

Stage or tissue	[illegible]	Rf [illegible]	[illegible]
In developing [illegible]			
G [illegible]		[illegible]	[illegible]
G [illegible]			
[illegible]			
G [illegible]			
G [illegible]		[illegible]	[illegible]
(post hatching)		[illegible]	[illegible]
		[illegible]	[illegible]
		[illegible]	[illegible]
		[illegible]	[illegible]
In adult			
Muscle	[illegible]	0.141	[illegible]
		[illegible]	[illegible]
		0.206	2+
		[illegible]	[illegible]
		[illegible]	[illegible]
Heart	[illegible]	0.141	[illegible]
		0.173	1
		0.206	2+
		0.233	3+
		0.282	1
Liver	[illegible]	0.062	3+
		0.141	3+
		0.173	1+
		0.206	[illegible]
		0.233	[illegible]
		0.282	[illegible]
Eye	[illegible]	0.141	2+
		0.173	1+
		0.206	1+
		0.233	2+
		0.282	[illegible]
Kidney	[illegible]	0.141	3+
		0.173	1+
		0.206	3+
		0.233	3+
		0.282	3+
Brain	[illegible]	0.141	2+
		0.173	2+
		0.206	2+
		0.233	2+
		0.282	2+

1+, faint staining (marginal presence); 2+, moderate staining (intermediate abundance); 3+, intense staining (most abundant).

In *Catla catla*, *Cirrhina mrigala* and *Labeo rohita*, 3 LDH bands were expressed in both pre- and post-hatching stages while 5 bands were observed throughout the developmental stages of *Labeo bata*[12]. However, during the initial stages of development, some species have been reported to express only one LDH band depicting B_4 isozyme (e.g. in trout[13]) while in some others, only A_4 is expressed (e.g. in *Micropterus salmonoides salmonoides*[14]). In others, both LDH A and B sub-units may be expressed equally[15]. It seems that the single LDH band appearing during the pre-hatching stages represented B_4 isozyme as indicated by the relative anodal mobility. The five bands expressed in both post-hatching stages and in adult tissues presumably represent five tetrameric associations, viz. A_4, A_3B_1, A_2B_2, A_1B_3 and B_4.

The additional lowly anodal band in the liver has presumably liver specific C gene expression. Incidentally, the appearance of LDH C_4 has been reported to coincide with either retinal development[16,17] in some species, where eye specific C gene expression occurs, or with liver embryogenesis in some others, where liver specific expression of the gene occurs[18]. In the present study, the LDH C gene expression is lacking in embryos even up to 72 h after hatching, presumably because functional activity of the liver did not start, though it had been formed. Further immunochemical studies may be helpful in confirming the genetic involvement speculated through the expression of the LDH bands.

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AEROMONAS HYDROPHILA SEPTICAEMIA OF INDIAN MAJOR CARPS IN SOME COMMERCIAL FISH FARMS OF WEST GODAVARI DISTRICT, ANDHRA PRADESH

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AEROMONAS HYDROPHILA is an important pathogen of warm water fishes[1]. Gopalakrishnan[2] reported many instances of entire populations of Indian major carps being wiped out by epidemics of *A. hydrophila* infection in stocking tanks in West Bengal, India. Snieszko and Axelrod[3] classified disease symptoms caused by *A. hydrophila* under four categories, viz. acute, rapidly fatal septicaemia, with a few gross symptoms; an acute form with dropsy, blisters, abscesses and scale protrusion; chronic ulcerous form with furuncles and abscesses; and latent form with no symptoms. An ulcerative form of *A. hydrophila* infection in *Catla catla*[4] has been reported earlier. An acute septicaemia due to *A. hydrophila* in some commercial fish farms of West Godavari District of Andhra Pradesh is discussed here.

Following complaints from some fish farmers in Eluru, West Godavari District, Andhra Pradesh, of mortality of fish in their farms during April 1988, infected fish were collected.

Farm A: Water spread area of 7 acres, stocking three Indian major carps, Catla, Rohu and Mrigal, in a ratio of 1:2:0.8. At the time of sampling, average weights were Catla 0.8 kg, Rohu 1 kg, Mrigal 0.5 kg. Mortality was noticed in Catla and Rohu at the rate of 6–7/day and 1–2/day respectively.

Farm B: Water spread area of 20 acres, stocking Catla, Rohu, Mrigal and grass carp in the ratio 1:3:0.5:0.125. Average weights of fish at the time of sampling were Catla 0.75 kg, Rohu 1.25 kg, Mrigal 1 kg and grass carp 2.0 kg. Mortality was noticed only in Rohu at the rate of 10–15/day.

Fish from both farms showed dark patches on the body. Live fish were transported to the laboratory for collection from surface lesions for culturing. Fish were anaesthetized by keeping cotton dipped in 70% alcohol under the operculum. The fish was then cut open using sterile instruments. Blood was drawn from the heart, and pieces of liver, kidney and spleen were removed, taking care to avoid contamination from the alimentary canal. All samples were plated on trypticase soy agar and incubated at ambient temperature. Isolates were purified and initial identification of the isolates was made using the diagnostic scheme suggested by Plumb and Bowser[5]. Identification of *A. hydrophila* was by a series of biochemical tests described earlier[4].

Surface swabs from infected fish yielded predominantly *A. hydrophila*. This organism was isolated in pure culture from blood, liver and kidneys of infected fish. This indicated that there was acute septicaemia due to *A. hydrophila*. The organism's presence in blood, liver and kidney is a clear indication of it causative role. In both farms, the species that was in larger number was affected.

Factors contributing to virulence of *A. hydrophila* have been investigated earlier[6]. Allan and Stevenson[7] demonstrated that crude extracellular preparations of *A. hydrophila* containing haemolytic and proteolytic activities could produce pathological symptoms in trout. Thune *et al.*[8] also demonstrated that crude extracellular preparations from *A. hydrophila* containing haemolysin and heat-stable

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†For correspondence

Table 1 *Virulence of A. hydrophila isolates from diseased carp*

Fish from which isolated	Site from which isolated	LD_{50}	Haemolytic activity	Protease production	MHD*
Farm A					
Rohu	Blood	1.3×10^6	+	+	10^8
	Liver	2.4×10^6	+	+	10^8
	Surface	3.1×10^6	+	+	10^8
Catla	Blood	3.6×10^5	+	+	10^7
	Liver	4.2×10^5	+	+	10^7
Farm B					
Rohu	Blood	1.4×10^5	+	+	10^7
	Surface	1.2×10^6	+	+	10^8

*Minimum number of bacterial cells required to bring about agglutination of catfish erythrocytes.

and heat-labile proteases could produce gross clinical signs similar to those produced by the whole organism. The isolates obtained in this study showed haemolytic and proteolytic activity (table 1), indicating their virulence. The LD_{50} of the isolates to catfish fingerlings ranged from 10^5 to 10^6 (table 1). De Figueiredo and Plumb[9] examined the virulence of *A. hydrophila* strains isolated from diseased fish to channel catfish fingerlings, and obtained LD_{50}s in the range 10^4–10^5. The LD_{50}s of our isolates to *Clarias batrachus* fingerlings were only marginally higher.

Haemagglutinating activity is closely related to the adhesive property of the organisms and is believed to play an important role in the infectivity of pathogens. Therefore we tested the isolates for haemagglutinins for catfish erythrocytes by the method of Toranzo *et al.*[10] As shown in table 1, all the isolates had haemagglutinins and the mean haemagglutination dose (MHD) ranged from 10^7 to 10^8 cells.

The present results show that mortality of Indian major carps noted during April 1988 in some fish farms is due to *A. hydrophila* septicaemia. Isolation of this organism from blood and internal organs of infected fish and the characterization of the isolates with respect to virulence factors like haemolysin, protease and haemagglutinins strongly support the conclusion regarding their causative role. The isolates were sensitive to tetracycline, cotrimaxazole and furazolidone.

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PHYSIOLOGICAL EFFECT OF MALATHION ON THE BRAIN OF *SAROTHERODON MOSSAMBICUS*

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THE sublethal effect of malathion on the metabolism of phospholipids in brain tissue, with which cerebral activity is quantitatively linked, was studied. Malathion toxicity depends on physicochemical properties, such as lipid solubility, which alters the permeability of the lipoprotein cell membrane of neurons and thus affects bioelectrical stimulation.

Sprague[1] considered the biochemical effects of pollution as basic. These can then be related to the efficiency of tissues and organs. According to Mason[2], sublethal effects may be observed at physiological, biochemical and behavioural levels. Investigation of the sublethal effects of malathion on brain physiology of *Sarotherodon mossambicus* should provide useful information.

In the present study, synthetic, biodegradable malathion (50 EC, manufactured by Bangalore Pesticide Ltd), used in agriculture and malaria control operations in the area, was chosen and bioassay tests were conducted. *S. mossambicus* (Peters) of average size 10 ± 1 cm was used. The fish, brought from local ponds, were acclimated in aquaria containing tap-water (pH 7.1). The medium was maintained at 85–100% oxygen saturation.

The static bioassay procedure outlined by Doudoroff *et al.*[3], with some modifications with regard to the laboratory techniques, was employed. Tests were conducted at room temperature, 29 ± 1°C. The specific period of exposure was 96 h. For carrying out the 10-day exposure tests, a batch of 10 fish was kept in 0.01 ml/l EC, which is two-thirds of the 96-h LC_{50} value. Another lot of 10 fish was kept in tap-water as control. The fish (treated and controls) were sacrificed after 5 and 10 days' exposure and the brains dissected out for colorimetric analysis.

The physical reactions of the fish to sublethal concentration of malathion are manifested in erratic swimming, convulsions, commencing with rapid, jerky movements of the body and fins, insensitivity to external stimuli, and weakening of respiration.

According to Corbett[4] the site of action of organophosphates is the enzyme acetylcholinesterase, which hydrolyses the synaptic transmitter acetylcholine. In the presence of an organosphosphate inhibitor, the enzyme is phosphorylated at its anion and esterase centres and cannot react with acetylcholine[5]. The likely consequence of acetylcholinesterase inhibition is the accumulation of acetylcholine, which is likely to cause prolonged excitatory postsynaptic potential. It might first lead to stimulation and later cause a total block in the cholinergic system. It might be due to this that fish initially show hyperactivity, followed by convulsive and uncoordinated movements, ending in paralysis. Table 1 shows that phospholipid content of brain tissue decreased in exposed fish while in controls it increased. The reduction was significant after 10 days of exposure.

Table 1 *Total phospholipid (μg/mg wet wt) in brain tissue of control and malathion-exposed S. mossambicus*

	5 Days	10 Days
Control	8.65 ± 0.24	9.31 ± 0.85
Malathion-exposed	7.79 ± 0.62	6.94 ± 0.27

Each value is mean ± SD of 5 individual observations.

Various workers[6–9] have reported that synthesis of lipids and proteins in brain cells is probably dependent on stimulation of neural tissue. Malathion being fat-soluble[4] might be binding to the nerve cell membrane. Its lipid solubility may be an active factor in altering the membrane permeability of neurons for the conduction of Na^+ and K^+ ions and thus interfering with bioelectrical stimulation. Grundfest[10] found that the bioelectric phenomenon results almost entirely from the permselective properties of the cell membrane, because, due to increased conductance, resistance decreases.

The reduction in phospholipids would retard the physiological activity of the central nervous system. Ramachandran *et al.*[11] observed that phospholipids play a vital role as constituents of neuron cell membrane and in regulating membrane permeability upon which bioelectrical stimulation depends. Phospholipids are also present in the myelin sheath of nerve cells and in electron transport particles. Therefore reduction of phospholipid synthesis in neural tissue might further inhibit neurotransmission. Balasundaram[12] observed that inhibition in neurotransmission can be taken as an indicator of dysfunction in other organ systems whose integration in metabolism is vital for survival. Thus the weakening of respiration might also be due to neurotransmitter failure.

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ACCUMULATION OF PROLINE AND POLY-β-HYDROXYBUTYRATE IN HIGH TEMPERATURE-RESISTANT STRAINS OF *AZOSPIRILLUM*

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WE have recently described the occurrence of high temperature-resistant strains (HTR) of the nitrogen-fixing associative symbiotic bacterium, *Azospirillum*[1,2], and suggested their use as biofertilizer for summer crops. In the tropics high soil temperature appears to limit biological nitrogen fixation (often soil temperature exceeds 50°C). Although the mechanism of thermophilism in bacteria has been fairly well understood[3], no information is available for *Azospirillum*, which is a typical mesophilic bacterium. Proline and poly-β-hydroxybutyrate (PBHP) have been implicated in response to temperature and moisture stresses in organisms[4,5]. We are reporting here the possible mechanism of high-temperature resistance in *Azospirillum*.

Of fifteen HTR strains from 70 isolates of *Azospirillum*, AZ.Ht.1 and AZ.Ht.2 were chosen for the study as these isolates registered fairly high nitrogen-fixing potential. A standard mesophilic strain, *A. brasilense* Sp. 7(ATCC 29145) (supplied by Dr J. Dobereiner, Brazil), was also included in the study for comparison.

To determine PBHB, the method of Zevenhuizen[6] was followed. *Azospirillum* strains were grown in 100 ml quantities of yeast extract glucose broth in 250 ml Erlenmeyer flasks at 30 and 50°C over a temperature-controlled environmental shaker. After five and ten days of incubation cells were harvested by centrifugation at 10,000 *g* for 15 min at 4°C. The cells were suspended in sterile water and one ml of the cell suspension was digested in one ml of 2 N hydrochloric acid at 100°C for 2 h in a steam chamber. After cooling, the digest was extracted with 2.5 ml of chloroform. Samples, containing lipids, were transferred to test tubes after evaporating the chloroform over a water bath. Five ml of 96% sulphuric acid was added and the samples were heated at 100°C for ten min in a steam jacket. The absorbance of the clear solution was recorded at 235 nm in a double-beam spectrophotometer. The PBHB content was calculated from the formula

Extinction coefficient $E_{235} = 0.35$ per 100 μg of PBHB.

To determine proline, the cells were similarly grown in yeast extract glucose broth at 30 and 50°C as described earlier and the procedure of Bates *et al.*[7] was followed for the determination. Colour development was achieved by the addition of acid–ninhydrin mixture (a mixture of 1.25 g of ninhydrin dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid).

The results are presented in tables 1 and 2. PBHB granules in *Azospirillum* are considered to be a storehouse of energy-rich material usable by the

Table 1 *Poly-β-hydroxybutyrate in Azospirillum isolates*

	5 Days' growth		10 Days' growth	
Isolate	30°C	50°C	30°C	50°C
AZ.Ht.1	0.387	1.908	0.990	3.085
AZ.Ht.2	0.789	2.163	1.209	5.835
Sp.7	0.859	0.834	0.886	0.709
Mean for 15 HTR isolates	0.944	1.654	1.107	3.421

PBHB in mg per g dry weight of cell. Each value is mean of three determinations.

Table 2 *Proline accumulation in Azospirillum isolates*

	5 Days' growth		10 Days' growth	
Isolate	30°C	50°C	30°C	50°C
AZ.Ht.1	64.0	104.0	89.6	136.0
AZ.Ht.2	32.0	192.0	69.1	284.0
Sp.7	68.0	78.0	74.2	91.2
Mean for 15 HTR isolates	61.8	96.8	74.6	112.8

Proline in μg per mg dry weight of cells. Each value is mean of three determinations.

organism at times of stress. PBHB content of HTR isolates AZ.Ht.1 and AZ.Ht.2 was higher than that of the standard mesophilic strain Sp.7. Also, PBHB content of the HTR strains was higher when the cells were grown at 50°C, while that of strain Sp.7 tended to decrease, presumably owing to the utilization of PBHB as a source of energy during the prolonged incubation. In general the 15 HTR isolates showed a spectacular increase in PBHB content at elevated temperature.

The HTR strains AZ.Ht.1 and AZ.Ht.2 strains also recorded sharp increase in proline content after growth at 50°C. In Sp.7 strain there was a slight increase in proline content at 50°C. All the 15 HTR isolates tended to accumulate proline at high temperature. The results suggest the role of PBHB and proline in temperature resistance of *Azospirillum* spp.

The problem of temperature sensitivity has been realized in the case of the root nodule bacterium *Rhizobium*. Eaglesham and Ayanaba[8] have indicated the occurrence of several strains of *R. trifolii* and *R. japonicum* which exhibited temperature tolerance up to 70–80°C. They have also stressed the need for developing HTR strains in *Rhizobium*. The occurrence of higher ratios of high-melting-point, saturated fatty acids to branched-chain fatty acids was reported to confer temperature tolerance to bacteria[9,10]. Also, intracellular accumulation of amino acids like proline and hydroxyproline[11], presence of thermostable proteins, and synthesis of heat shock proteins[12] have been suggested as possible mechanisms of high-temperature tolerance in microorganisms.

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9 September 1988

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IN VITRO EFFECTS OF THREE ORGANOPHOSPHORUS INSECTICIDES ON KINETIC CONSTANTS OF ACETYLCHOLINESTERASE IN A FRESHWATER TELEOST, *CLARIAS BATRACHUS* (LINN.)

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IT is well known that acetylcholinesterase (AChE, E.C. 3.1.1.7) is the target enzyme of both organophosphorus (OP) and carbamate pesticides[1,2]. The inhibition of AChE disrupts the transmission of the nerve impulse in the central and peripheral nervous system in vertebrates[3–5]. Several attempts were made to correlate the toxic action of OP pesticides with AChE inhibition and thereby to estimate their anticholinesterase activity[6–8]. OP and carbamate compounds inhibit cholinesterases by binding covalently to a serine residue in the active site of the enzyme[9]. Fish brain AChE activity can serve as a monitor of pollution by OP insecticides[10, 11]. The *in vitro* inhibition kinetics of AChE by trichlorfon, malathion and methylparathion (technical grade OP compounds) in brain and muscle tissues of the

Table 1 *Effect of trichlorfon, malathion and methylparathion on kinetic parameters of Clarias batrachus acetylcholinesterase*

Kinetic parameter	Tissue	Control	Trichlorfon	Malathion	Methyl parathion
V_{max} (μmoles of ACh hydrolysed/ mg protein/h	Brain	5.9	5.2 (−11.86)	5.5 (−6.78)	5.7 (−3.39)
	Muscle	3.7	3.0 (−18.92)	3.2 (−13.51)	3.5 (−5.41)
K_m	Brain	3.3	6.8 (+106.06)	5.0 (+51.52)	3.8 (+15.15)
(μM)	Muscle	2.9	5.1 (+75.86)	4.3 (+48.28)	3.7 (+27.59)

Values in parentheses indicate per cent change over control.

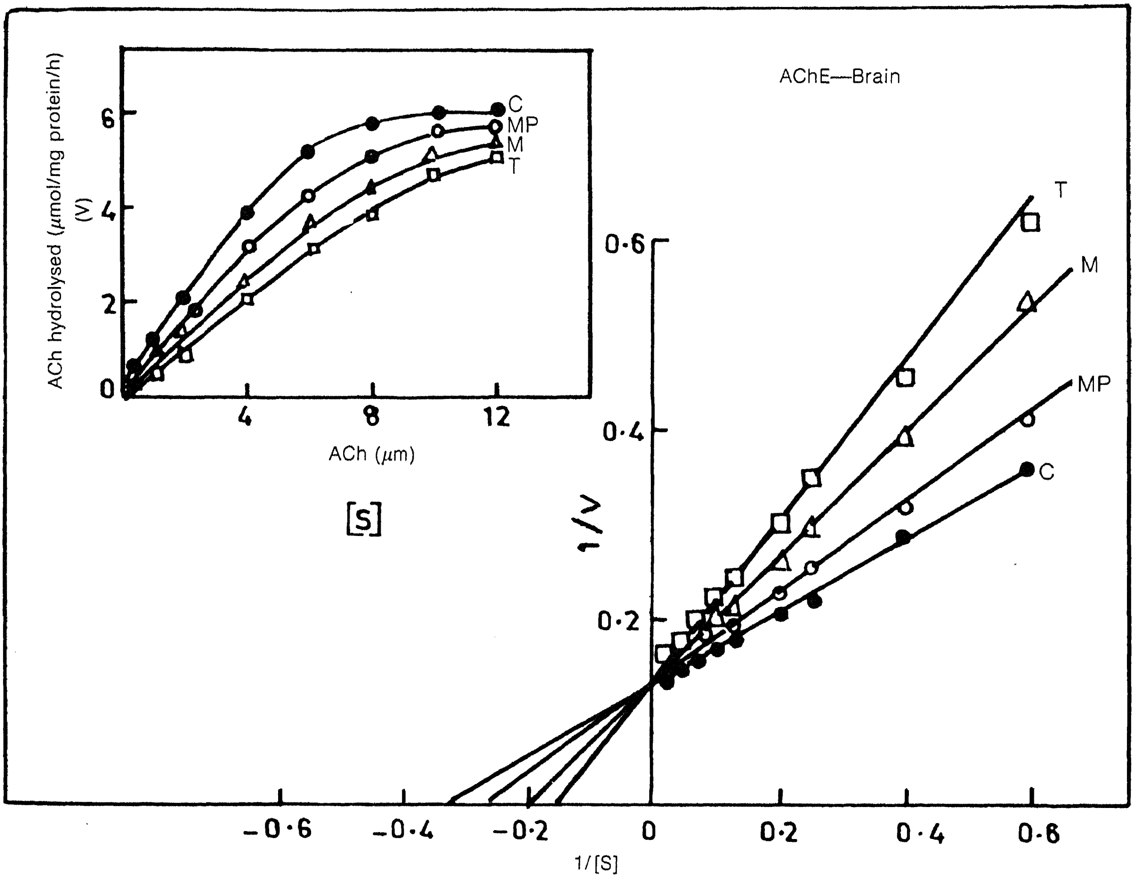

Figure 1. Lineweaver–Burk plots for *C. batrachus* brain AChE assayed in absence (●——●) and in presence of trichlorfon (□——□), malathion (△——△) and methylparathion (○——○). Inset, substrate concentration vs reaction velocity curves.

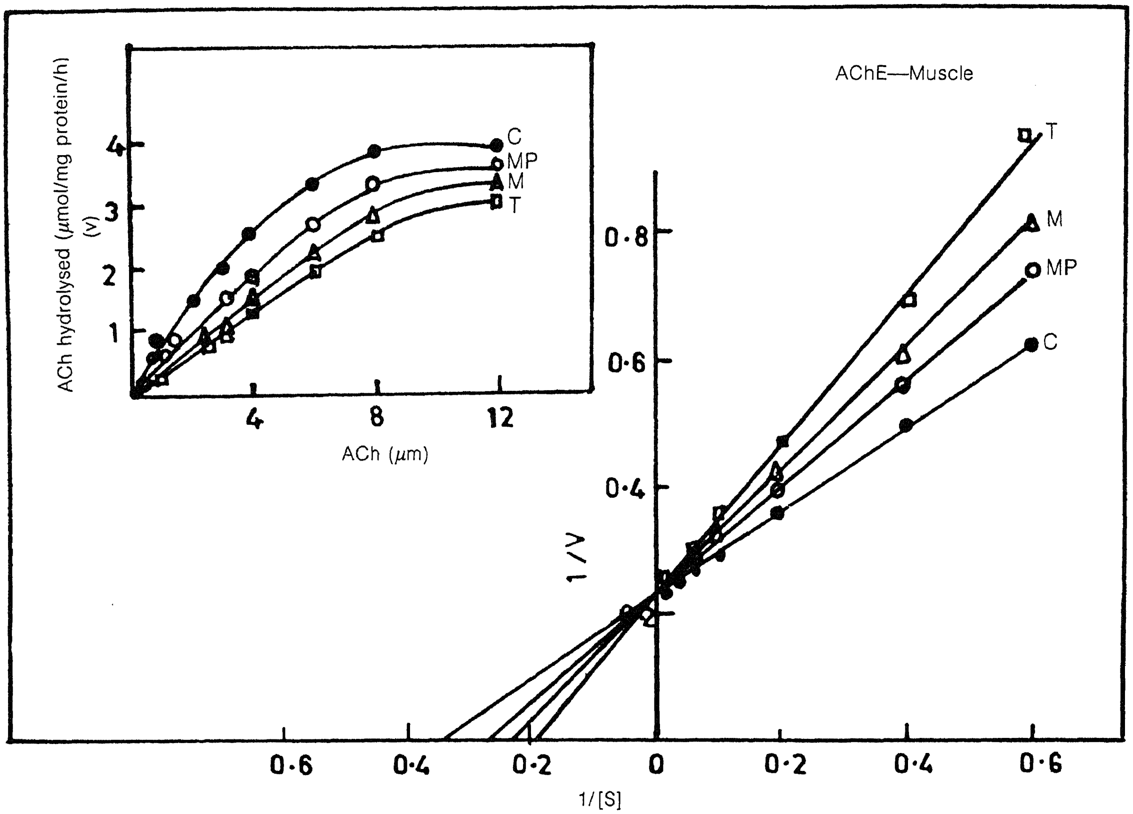

Figure 2. Lineweaver–Burk Plots for *C. batrachus* muscle AChE. See legend to figure 1 for details.

freshwater edible fish *Clarias batrachus* (Linn.) have been studied.

C. batrachus weighing 30–40 g and 20 cm in length were collected from the local market and acclimatized to laboratory conditions for about two weeks. They were fed *ad libitum* with groundnut cake and deprived of food before the day of experiment[12]. AChE was assayed by the method of Metcalf[3] in the presence of different concentrations (2–20 μmol) of trichlorfon, malathion and methylparathion individually to determine the IC_{50} values. Enzyme activity was expressed as μmoles of ACh hydrolysed/mg protein/h. Protein was determined according to Lowry *et al.*[13] The double reciprocal plots were prepared[14], and V_{max} and K_m values were calculated.

Fifty per cent inhibitory concentrations (IC_{50}) for trichlorfon, malathion and methylparathion on brain AChE were 4, 10 and 12 μM; in the case of muscle AChE the IC_{50} values were 8, 12 and 15 μM respectively. The effects of the pesticides on V_{max} and K_m of brain and muscle AChE are presented in table 1 and figures 1 and 2. In all cases there was significant increase in K_m values but no significant change in V_{max} values. Increase in K_m suggests decreased affinity of the enzyme for the substrate and also decreased rate of breakdown of the enzyme–substrate complex. The three OP compounds studied exert a competitive type of inhibition on AChE. Competitive inhibitors[15,16] are known to compete for the same active site with the substrate, resulting in the competitive type of inhibition. Most of the OP insecticides have structural analogy with the ester part of acetylcholine and bind to the esterase part of AChE[17].

One of the authors (VJS) thanks CSIR, New Delhi, for a fellowship.

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ANNOUNCEMENT

EVOLUTIONARY BIOLOGY WORKSHOP

A one-week workshop titled 'Topics in Evolutionary Biology' will be held at the Indian Institute of Science from 11 to 15 December (both days inclusive) this year. The subjects to be covered, at a level aimed at students as well as research workers, will be varied and will include optimization strategies, sexual selection, the evolution of insect sociality and the evolution of complexity. Intending participants should write to V. Nanjundiah, Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560 012, with their CV and a brief but specific statement of purpose indicating their interest. The **last date** for receipt of applications is **7 November 1989**. Those selected will be paid return second class train fare to Bangalore and back and a daily allowance sufficient to cover their stay at the IISc campus.

BOOK REVIEWS

Diseases of Sugarcane: Major Diseases, (eds) C. Ricaud, B. T. Egan, A. G. Gillaspie Jr. and C. G. Hughes, (Published by Elsevier Scientific Publishers, Amsterdam, The Netherlands), Price: Dfl. 250.

The International Society of Sugarcane Technologists (ISSCT) brought out two volumes on 'Sugarcane Diseases of the World'. The first volume appeared in 1961, and the second one in 1964. The publication reviewed here is the updated version of the first volume, with some alterations. In this book three new chapters, on, rust, grassy shoot and white leaf diseases, and plant quarantine, have been added, while three chapters in the earlier version, on *Fusarium* sett rot, root rot, and *Scleropthora* disease, have been deleted as the editors felt that these diseases are now of minor importance.

The book has 22 chapters and a collection of coloured photographs. These chapters have been written by individuals with vast experience in the field of their specialization. The introductory chapter is devoted to sugarcane—its botany and physiology—and highlights those aspects of sugarcane that are of utmost importance to sugarcane workers. The physiology of sugarcane has been dealt with in a masterly fashion.

The remaining 21 chapters have been divided into four parts. The first part deals with bacterial diseases like gummosis, leaf scald, ratoon stunting and red stripe. The second part covers fungal diseases like brown stripe, downy mildew, eye spot, leaf scorch, pineapple disease, Pokkah boeng, red rot, smut, rust and yellow spot. The third part deals with viral diseases like chlorotic streak, mosaic, Fiji, streak and grassy shoot and white leaf diseases. The fourth part (general) has only two chapters, one on sugarcane quarantine and the other on sugarcane diseases and their world distribution. Each chapter on diseases has been suitably divided into introduction, and sections on symptomatology, causal organism, epidemiology (survival, transmission, role of environmental factors, host range, etc.), economic importance and control, and at the end of each chapter a Spanish summary is also provided.

Some of the unique features of the book are: (i) Latest techniques have been described to aid the diagnosis of a disease. (ii) Recent taxonomic development has been taken into account to describe pathogens, and to avoid possible confusions, equivalent names are given in parenthesis. (iii) Excellent illustrations in the form of black and white photographs as well as colour photographs and beautiful camera lucida drawings have been included. (iv) Complete references instead of only abbreviated ones are provided.

There are also some shortcomings in the book: (i) The book does not cover some of the important findings of Indian research workers; for example, the Indian work on leaf scald has been completely left out; similarly, some of the important findings on red rot, pineapple disease and smut have also not been included. (ii) The taxonomic details of perfect stage of *Colletotrichum falcatum* have not been provided; this is essential in view of the existing controversy between *Physalospora tucumanensis* and *Glomerella tucumanensis*. (iii) Grassy shoot and white leaf diseases have been classified under viral disorders, which is not proper as the causal organisms of these diseases are supposed to be mycoplasma-like organisms. The usefulness of the book would have been even greater if a chapter on wilt, which is a severe problem in south-east Asia, had been included.

The cost of the book is Dfl 250.00 (= Rs. 2,000.00), which is quite exorbitant. It is not only beyond the reach of Indian research workers but also of small libraries. However, it is a welcome addition to big libraries of universities and research organizations. The editors are to be congratulated for elegant editing and for bringing out this informative publication on sugarcane pathology.

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Ecophysiology of Desert Vertebrates, (eds) P. K. Ghosh and Ishwar Prakash, (M/s Scientific Publishers, Ratananda Road, Maan Bhawan, P. O. Box 91, Jodhpur 342 001), 1988, pp. 475, Price: Rs. 425.

The desert ecosystem, despite its natural adversities, supports a good variety of fauna and flora.

Life in the desert calls for an array of adaptations befitting the characteristic, demanding environment. A wide range of adaptations, from mere classical behavioural patterns to highly synchronized physiological events, are a prerequisite to desert life.

Several books have been published in the general field of desert biology. Most of the contributions either include 'natural history' observations on individual desert species or project the environmental profile offered by this peculiar life-support system. For a long time a need has been felt for a compilation covering the ecological as well as functional aspects of desert animals, and the present book fulfils this need to some extent. The book contains articles from many authors and gives a fair idea of the strategies adopted by a few desert vertebrates in colonizing harsh habitats.

Twelve useful articles are included in the book. Except one, in which the physiological adaptation to cold deserts is dealt with in a general manner, the articles provide information on the life-style of one or the other vertebrate in relation to its environmental complex. The article on the role of dung beetles in relation to a herbivore community inhabiting an African desert environment is highly interesting and projects the important role played by these frail, detritivorous arthropods in nutrient cycling, soil elevation, aeration and water penetration. Of the three contributions pertaining to Indian deserts, one refers to vertebrate remains from archaeological sites, the second is on the haematology of the Indian camel, and the third details the ecological characteristics of two wild ungulate species populations. Inclusion of more examples from Indian deserts would have been appropriate and useful to Indian readers.

All the contributors have provided interesting and comparative information and the book would be a valuable addition to institutional libraries.

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Perspectives in Phytopathology, Dr R. S. Singh Festschrift volume, (eds) V. P. Agnihotri, N. Singh, H. S. Chaube, U. S. Singh and T. S. Dwivedi, (Published by Today and Tomorrow's Printers and Publishers, 24B/5, D. B. Gupta Road, Karol Bagh, New Delhi 110 005), 1989, pp. xiii + 523, Price: Rs. 595.00; $85.00.

This Festschrift incorporates original research papers as well as review papers contributed by distinguished scientists from all over the world, reflecting the reputation of Dr R. S. Singh, who has contributed immensely to the important field of plant pathology, and to the study of the fascinating soil-borne pathogens in particular.

The volume has 35 papers providing the latest information on both fundamental and applied aspects of plant pathology. The subjects have been chosen with care. One paper emphasizes various facets of the use of fungicides, an essential component of inputs for increasing plant productivity without which it is difficult to control many important diseases in modern agriculture. Other papers are devoted to immunological, ELISA and dot blot hybridization techniques for the detection as well as characterization of mycoplasmas, viruses and viroids respectively. The role of plasmids in phytopathogenic bacteria and the molecular mechanism of pathogenicity are the subject of another paper. Subsequent papers deal with mycotoxins, including aflatoxin, which has caused considerable alarm in recent years; biotrophic plant parasites and symbionts in tissue culture, focusing attention on techniques for the establishment of dual culture of biotrophic fungi and nematodes as well as their hosts; and screening germplasm of a wide variety of crops, such as cereals, legumes, oilseeds and medicinal plants, to salvage varieties resistant to various diseases.

Of special significance are the papers pertaining to bunts and smuts, the most destructive diseases of wheat and rice not only in India but in large parts of the world. These papers highlight the epidemiology of the diseases and the mode of perpetuation of the pathogens, and contain high-quality illustrations. The papers on diseases of economic significance caused by fungi, bacteria, viruses and mycoplasmas in cotton, cowpea, pigeonpea, triticale, coconut, potato, tomato and safflower are up-to-date and consolidated accounts. They contain information on the occurrence, physiological specialization and variation of the pathogens, disease cycle, epidemiology, disease resistance, host range, yield losses and disease management methods. Other papers examine biological control and soil solarization for the control of many plant diseases as alternatives to the excessive use of hazardous pesticides.

A clear message is that there is ample proof of ability to safeguard the interest of and protect Indian agri-horticulture from exotic pests and diseases by adopting suitable plant quarantine measures while importing a large variety of germplasm and planting material from different parts of the world with a view to incorporating desired genes and improving existing varieties.

Although the book has many glaring spelling mistakes most of the papers are good. The book is expensive, but certainly a fine volume for students, teachers and plant pathologists.

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Brief notes on books

We hope to publish brief notes on books that may interest readers. We invite readers also to contribute.
—Editor

The Papers of Thomas A. Edison, vol. 1, edited by R. Jenkins and others, Johns Hopkins University Press, 708 pp., $65.

Funded by two government agencies, two individuals, one labour union and forty-three corporations this is the first of a twenty-volume project that will finally emerge in the form of 16,000 large printed pages containing 7000 documents culled from a cache of a quarter million original sketches and three million pages of documents left by one of the most prolific inventors humanity has ever produced. Price of the entire set 1300 US dollars, weight 35 kg, shelf space of one metre!

Inventions are of two types—those like the cotton gin, the reaper, the telegraph, the telephone, the aeroplane or the lowly sewing machine, which heralded technological breakthroughs and are in a minority; and those that are just improvements or variations of existing technologies. Edison had inventions of both types—the incandescent lamp, the phonogram, the storage battery and motion pictures were instruments of change and therefore belong to the first type, while others, which are in this book, represent his early period and belong to the latter class.

Yes, he had a plethora of ideas: 'I have also in the process of manufacture 12 Universal Printing Machines, a Regulating Temperature Machine, Two other Perforating Machines, a New screw slotting machine, a wire straightening Machine, Polishing Machine and other things which also takes thinking.'

This edition retains the inventor's idiosyncratic punctuation, capitalization and his sometimes atrocious grammar and spelling.

We are told that headnotes are provided for sketches and the commentary is lavish. Each chapter is preceded by a biographical sketch illustrated with contemporary prints and photos and Edison's own autobiographical notes of this period are also reproduced—what a feast!

All the depository libraries of India must have copies of this remarkable set of books for young hopefuls to thumb through and see how an unusually inventive mind works.

We read with interest the review, in *New Scientist*, of the remarkable book *Molecular Sieves: Principles of Synthesis and Identification* (by Rosemarie Szostak Van-Nostrand Reinhold, 1989, pp. 524, £ 53.95), by L. V. C. Rees, Professor in the Department of Chemistry, Imperial College, London.

The following remarks he makes would be of some interest to Indian scientists.

'I received *Molecular Sieves* (for review) just before departing on a three-week visit to the National Chemical Laboratory in Puna [*sic*], India. The laboratory has a large group working on the synthesis of many materials discussed by Szostak. The value of her book is exemplified by the fact that it was in continuous demand by all members of the group—I saw little of it during my stay.'

It is strangely coincidental that some of the best natural zeolites of India are to be found near Pune.

THE SCIENTIFIC COMMUNITY

1989 INSA Awards

Satyendranath Bose Medal to Prof. Chanchal Kumar Majumdar, Director, S.N. Bose National Centre for Basic Sciences, Calcutta—for work in statistical physics.

Jagadish Chandra Bose Medal to Prof. N. Appaji Rao, Dept of Biochemistry, Indian Institute of Science, Bangalore—for work in enzyme regulation and population studies of inborn errors of metabolism.

Darshan Nosherwanji Wadia Medal to Prof. Saurindra Nath Sen, Dept of Geology, Calcutta University, Calcutta—for structural and tectonic interpretation of the Precambrian rocks of Rajasthan.

Syed Hussain Zaheer Medal to Prof. Rajinder Kumar, Dept of Chemical Engineering, Indian Institute of Science, Bangalore—for contributions to analysis of multi-phase systems and design of a very large commercial fluidized bed reactor.

Golden Jubilee Commemoration Medal in Chemical Sciences to Prof. Goverdhan Mehta, Dean, School of Chemistry, University of Hyderabad, Hyderabad—for work in synthesis of strained polycyclic systems and complex bioactive terpenoids.

Golden Jubilee Commemoration Medal in Biological Sciences to Prof. John Barnabas, Head, Division of Biochemical Sciences, National Chemical Laboratory, Pune—for work in molecular evolution.

INSA–Vainu Bappu Memorial Award to Prof. Geoffrey Burbidge, University of California, San Diego, USA—for contributions to understanding of stellar nucleosynthesis, extra-galactic radio sources, high energy astrophysics and quasistellar objects.

INSA Prize for Materials Science to Prof. P. Rama Rao, Director, Defence Metallurgical Research Laboratory, Hyderabad—for contributions to physical and mechanical metallurgy, particularly titanium metallurgy.

Professor L. S. S. Kumar Memorial Award to Dr Daman Saluja, Biochemistry and Molecular Biology Lab., Dept of Botany, University of Delhi, Delhi—for contributions in plant science.

Bashambar Nath Chopra Lecture to Dr Pushpa Mittra Bhargava, Director, Centre for Cellular and Molecular Biology, Hyderabad—for work on cell growth regulation and seminal plasma.

Professor K. Rangadhama Rao Memorial Lecture to Prof. Mihir Chowdhury, Indian Association for the Cultivation of Science, Calcutta—for work on two-photon processes in biomolecules and dynamics of excited state proton transfer.

Dr D. P. Chatterjee Memorial Lecture to Prof. Ramamurthi Rajaraman, Centre for Theoretical Studies, Indian Institute of Science, Bangalore—for work in a variety of areas in theoretical physics, particularly solitons.

Professor Panchanan Maheshwari Memorial Lecture to Prof. Vishwambar Puri, Professor–Emeritus, Institute of Advanced Studies, Meerut University, Meerut—for contributions in floral morphology.

Dr Nitya Anand Endowment Lecture to Dr Chhitar Mal Gupta, Head, Division of Membrane Biology, Central Drug Research Institute, Lucknow—for work on biomembrane structure and new drug delivery systems.

A.K. Bose Memorial Award (to a recipient of INSA Young Scientist Medal) to Dr Dhruba Jyoti Biswas, Bhabha Atomic Research Centre, Bombay—for work on nonlinear-dynamical behaviour in laser systems.

Hari Om Awards

The UGC has selected ten persons for the Hari Om Ashram Trust Award for 1985 and 1986.

C. V. Raman Award for research in physical sciences to Prof. M. V. George, Indian Institute of Technology, Kanpur, and Prof. J. C. Bhattacharyya, Indian Institute of Astrophysics, Bangalore.

Homi J. Bhabha Award for research in applied sciences to Prof. P. Rama Rao, Defence Metallurgical Research Laboratory, Hyderabad, and Dr K. L. Chopra, Indian Institute of Technology, Kharagpur.

Meghnad Saha Award for research in theoretical sciences to Prof. N. Mukunda, Indian Institute of Science, Bangalore, and Prof. R. P. Bambah, Vice-Chancellor, Punjab University, Chandigarh.

J.C. Bose Award for research in life sciences to Prof. V. Sasisekharan, Indian Institute of Science, Bangalore, and Prof. M. K. Chandrasekharan, Madurai Kamaraj University, Madurai.

Awards in the field of interaction between science and technology and society to Prof. B. M. Udgaonkar, Tata Institute of Fundamental Research, Bombay, and Dr Saroj Ghose, National Council of Science Museums, Calcutta.

The Central Board of Irrigation and Power Jawaharlal Nehru Birth Centenary Year Research Award 1988 was given to Mr Rajesh Chandra, (presently at) Technical Liaison Mission of the Department of Atomic Energy in Paris (formerly Head, Fuel Handling Systems, Reactor Engineering Division, Bhabha Atomic Research Centre, Bombay), for services rendered to the development of nuclear power in India.

UGC Emeritus Fellowships

Prof. L. K. Mahapatra, Utkal University, Bhubaneswar—anthropology; Prof. H. C. Arya, Rajasthan University, Jaipur, and Dr M. M. Bhandari, Jodhpur University, Jodhpur—botany; Prof. M. R. Krishnamurthy, Regional Engineering College, Tiruchirapalli—computer science; Prof. K. S. Viswanathan, Kerala University, Trivandrum, Dr S. Lokanathan, Rajasthan University, Jaipur, and Prof. B. S. Sood, Punjabi University, Patiala—physics; Prof. H. K. Singh, Punjab University, Chandigarh—pharmaceutical science; Prof. B. K. Tikkiwal, Rajasthan University, Jaipur—statistics; Prof. H. B. Devaraj, Mysore University, Mysore—zoology; Prof. P. C. Pandeya, Jiwaji University, Gwalior—environmental science.

B. P. Pal passes away

Benjamin Peary Pal, well-known agricultural scientist and former Director-General of the Indian Council of Agricultural Research, died on 14 September. Prof. Pal made many important contributions in plant breeding. He was a fellow of the Royal Society, Indian Academy of Sciences and Indian National Science Academy, Honorary Fellow of the Linnaean Society, and foreign member of the Genetic Society of Japan and Academie Agriculture de France.

ANNOUNCEMENT

INTERNATIONAL UNION OF THEORETICAL AND APPLIED MECHANICS

IUTAM Congress

The International Congresses, organized every four years by IUTAM, bring together scientists from all over the world for the purpose of exchanging information about recent developments in the field. Participation is unrestricted.

18th International Congress on Theoretical and Applied Mechanics

Place: Haifa, Israel
Date: 23–29 August 1992
Contact: Prof. Werner Schiehlen
Secretary-General, IUTAM
University of Stuttgart
Pfaffenwaldring 9
D-7000 Stuttgart 80, FRG

IUTAM Symposium calendar

The IUTAM Symposia are reserved to invited participants. Those wishing to participate in an IUTAM Symposium are therefore advised to contact the Chairman of the Scientific Committee under the address given below far in advance of the meeting.

Symposia 1990

IUTAM Symposium on Mechanical Modelling of New Electromagnetic Materials

Place: Stockholm, Sweden
Date: 2–6 April 1990
Chairman: Prof. R. K. T. Hsieh
Department of Mechanics
Royal Institute of Technology
S-10044 Stockholm, Sweden

IUTAM Symposium on Dynamical Problems of Rigid-Elastic Systems and Structures

Place: Moscow, USSR
Date: 23–27 May 1990
Chairman: Prof. D. M. Klimov
Institute for Problems of Mechanics
Pr. Vernadskogo 101
Moscow 117 526, USSR

IUTAM Symposium on Inelastic Deformation of Composite Materials

Place: Troy, USA
Date: 29 May–1 June 1990
Chairman: Prof. G. J. Dvorak
Rensselaer Polytechnic Institute
Troy, NY 12180, USA

IUTAM Symposium on Dynamics of Marine Vehicles and Structures in Waves

Place: Uxbridge, England
Date: 25–27 June 1990
Chairmen: Prof. R. E. D. Bishop
Brunel University
Uxbridge, Middlesex UB8 3PH, UK

Prof. W. G. Price
Department of Mechanical Engineering
Brunel University
Uxbridge, Middlesex UB8 3PH, UK

IUTAM Symposium on Separated Flows and Jets

Place: Novosibirsk, USSR
Date: 9–13 July 1990
Chairman: Prof. V. G. Dulov
Institute for Theoretical and Applied Mechanics
630 090 Novosibirsk, USSR

IUTAM Symposium on Fluid Mechanics of Stirring and Mixing

Place: San Diego, USA
Date: 20–24 August 1990
Chairmen: Prof. H. Aref
Institute of Geophysics and Planetary Physics
A-025, UCSD
La Jolla, CA 92093, USA

Prof. J. M. Ottino
159 Goessmann Lab.

Department of Chemical Engineering
University of Massachusetts at Amherst
Amherst, MA 01003, USA

IUTAM Symposium on Local Effects in Thin-Walled Structures

Place: Prague, Czechoslovakia
Date: 4–7 September 1990
Chairman: Prof. V. Krupka
Institute of Applied Mechanics
Hroznova 53
Cs-60300 Brno 3, Czechoslovakia

IUTAM Symposium on Nonlinear Hydrodynamic Stability and Transition

Place: Valbonne, France
Date: 4–8 September 1990
Chairman: Prof. G. Iooss
I.M.S.P., Université de Nice
Parc Valrose
F-06034 Nice, France

Fourth IUTAM Symposium on Creep in Structures

Place: Cracow, Poland
Date: 10–14 September 1990
Chairman: Prof. M. Zyczkowski
Politechnica Krakowska
ul. Warszawska 24
31–155 Krakow, Poland

Constitutive Relations for Finite Deformation of Polycrystalline Metals

Date: open
Place: Beijing, China

IUTAM Symposia 1991

Numerical Simulation of Nonisothermal Flow of Viscoelastic Liquids
27–31 May 1991; Kerkrade, The Netherlands

Mechanical Effects of Welding
10–14 June 1991; Lulea, Sweden

Nonlinear Stochastic Mechanics
1–5 July 1991; Turin, Italy

Breaking Waves
15–19 July 1991; Sydney, Australia

Finite Inelastic Deformations
19–23 August 1991; Hannover, FRG

Interpretation of Time Series from Mechanical Systems
26–30 August 1991; Warwick, England

Aerothermodynamics in Combustors
Date: open Place: Taipei, Taiwan

Microgravity Fluid Mechanics
Date: open Place: Bremen, FRG

Local Mechanics Concepts for Composite Materials System
Date: open Place: Blacksburg, USA

Mechanics of Fluidized Beds
Date: open Place: Stanford, USA

Edited and published by Prof. S. Ramaseshan, Current Science Association, Bangalore 560 080.
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COMMENT

COLD FUSION PRODUCES MORE TRITIUM THAN NEUTRONS

FLEISCHMANN *et al.*[1] reported the occurrence of nuclear fusion during the electrolysis of 99.5% D_2O (containing 0.1 M LiOD) using a palladium cathode. They found emission of neutrons, accumulation of tritium in the electrolyte (as a result of electrolysis), and generation of excess heat over and above that due to the following electrode reactions:

cathode reactions.

$$D_2O + e \rightarrow D_{ads} + OD^- \tag{1}$$

$$D_{ads} + D_2O + e \rightarrow D_2 + OD^- \tag{2}$$

$$D_{ads} + D_{ads} \rightarrow D_2 \tag{3}$$

balancing anodic reaction

$$4\,OD^- \rightarrow D_2O + O_2 + 4\,e. \tag{4}$$

The net heat due to the reactions (1), (2), (3) and (4) is zero, and these reactions contribute a cell voltage of 1.54 V, which has been referred to, by these authors, as the voltage for the above thermoneutral reactions. In addition they also observed the emission of γ-rays from the electrolytic cell. To explain these observations, they proposed the occurrence of the following nuclear (fusion) reactions in the Pd cathode:

$${}^{2}D + {}^{2}D \rightarrow {}^{3}T\,(1.01\ \mathrm{MeV}) + {}^{1}H\,(3.02\ \mathrm{MeV}) \tag{5}$$

$${}^{2}D + {}^{2}D \rightarrow {}^{3}He\,(0.82\ \mathrm{MeV}) + n\,(2.45\ \mathrm{MeV}). \tag{6}$$

The generation of γ-rays was explained to be due to the (n, γ) reaction

$${}^{1}H + n\,(2.45\ \mathrm{MeV}) \rightarrow {}^{2}D + \gamma\,(2.5\ \mathrm{MeV}). \tag{7}$$

At about the same time Jones *et al.*[2] reported (based on their observation of neutron emission) cold fusion in the electrolysis of D_2O (99.5%) containing a mixture of salts, with a titanium cathode.

These reports have created great stir among various research groups all over the world. As a result, there have been many confirmations and denials. However, only a few of the confirmations have been published in scientific journals. Reports of failure to duplicate the results of Fleischmann *et al.* have been discussed in the special meetings organized to understand cold fusion but most of them have not been published. Mathews *et al.*[3] found a significant amount of neutron emission over the background in the electrolysis of D_2O (containing nickel and palladium chloride) with Ti cathode. They also reported that the cathode was hotter in D_2O electrolysis compared to H_2O electrolysis. Santhanam *et al.*[4] reported production of excess heat (over and above that of the electrode reactions) in the electrolysis of 1 M NaCl in D_2O. Electrolysis using a Pd cathode yielded greater excess heat compared to electrolysis using a Ti cathode. γ-Rays were also detected during electrolysis. The radiation level was 48% higher than the background in one experiment while it was only 8% more than the background in another experiment. The Central Electrochemical Research Institute, Karaikudi, came up with a confirmation of cold fusion[5] based on calorimetric measurement and detection of tritium (by autoradiography) embedded in the Pd cathode.

Confirmation of cold fusion was reported by Scaramuzzi at the workshop on cold fusion held at Santa Fe (May 23–25)[6]. This was a different type of experiment, carried out at Frascatti. The logic for doing this experiment appeared to be that, in the electrolysis experiment, cold fusion was the result of interaction of D atoms in the metal lattice, and the role of electrolysis was to load the metal with D_2. In the light of this argument they loaded Ti pieces with D_2 gas from a D_2 gas bottle at 8 atm pressure and liquid nitrogen temperature. When the D_2-loaded Ti was heated to 200°C, with simultaneous evacuation, there was emission of neutrons. Also reported was the work of Magni of the ENFA CASSACCIA, who used a sponge of Ti loaded with D_2, which was cooled and then pumped. A small peak of neutrons was observed after about 700 min. Bursts of neutrons were also observed by Massoni *et al.* when blades of Ti (0.5 mm thick) were heated quickly (20 s) to 1000°C and cooled in gaseous D_2 to 500°C.

Recent results of several research groups of the Bhabha Atomic Research Centre (BARC), Bombay, reported by Dr P. K. Iyengar[7], reveal new and interesting features of cold fusion. Electrolytically induced cold fusion has been investigated by BARC employing a Milton–Roy cell (a commercial cell used for production of H_2) with Pd–Ag tubular cathode, and cells made in the laboratory with Pd cathode. They monitored both production of neutrons and accumulation of tritium. Both BF_3 detector embedded in paraffin and proton recoil fast neutron detector showed substantial neutron emission. In the case of electrolysis using the Milton–Roy cell, the BF_3 counter measured 17,758 counts/300 s (background 65 counts/300 s) and the proton recoil neutron detector gave a count rate of 25,872 counts/300 s (background 650 counts/300 s). Taking into account the efficiency of the counters, the neutron emission rate was estimated to be $\sim 2 \times 10^7$ neutrons/300 s. Neutron emission was not continuous but occurred in bursts, and the duration of the 'flashes' was probably under 160 μs. The emission was basically Poisson in nature (i.e. the neutrons were emitted one at a time).

BARC, unlike many other groups, carefully monitored tritium levels in electrolytes, taking precautions to use ^{40}K-free vials to minimize background counts and minimizing chemiluminescence interference effects. Excess tritium produced by electrolysis was computed, taking into account initial concentration of tritium and dilution effects due to periodic make-up of D_2O. Their estimates should be regarded as conservative because tritium locked in the cathode and tritium lost in electrolysis were neglected (it is known that the T/D ratio in gas evolved from the cell is nearly the same as that in the electrolyte). Typical results for tritium enrichment are shown in table 1. On the basis of a comparison between the neutron and tritium production rates, it is reported[7] that cold fusion may be characterized as being essentially 'aneutronic', with a neutron-to-tritium channel branching ratio of less than 10^{-8}. This is a surprising result when viewed in the light of the fact that muon-catalysed D–D fusion has a channel branching ratio of nearly unity (T and n production probabilities are 0.42 and 0.58 respectively[8]). Viewing the occasional burst of 100 neutrons in a single burst in the light of the branching ratio estimate of 10^{-8}, the report[7] states an intriguing conclusion, viz. that a chain reaction involving as many as 10^{10} fusion reactions must be occurring within 160 μs.

BARC reproduced with success[7] the Frascatti experiment. Ti pieces were exposed to D_2 gas at 50 atm. When this charge was subjected to temperature cycling between 77 K and 300 K emission of neutrons was noticed. It was not constant but exhibited several peaks. Typical count rate for a peak was 3900/40 s (background 60/40 s). In a 7 h duration the neutron emission was $\sim 6.5 \times 10^6$ neutrons. In another experiment D_2 gas was loaded into small Ti machined targets (discs, cones, etc.) at 1 atm pressure and 900°C (temperature attained by induction heating). Switching off the induction heating cooled the charge, and Ti absorbed D_2 gas in a minute. The targets showed statistically significant excess neutrons (over the background) during the gas absorption. One of the discs emitted neutrons nearly 50 h after loading and produced in all $\sim 10^6$ neutrons during an 85 min active phase.

Perhaps the most significant experiment carried out by BARC was that of establishing the presence of tritium in Ti loaded with D_2 gas. If D–D fusion took place in gas-loaded Ti, atoms of tritium should be produced and they must be locked up in the Ti lattice. Autoradiography of deuterated Ti discs showed randomly distributed intense spots, indicating that tritium was not uniformly distributed but was segregated in small regions that were random in location.

Another experiment for confirming the presence of tritium in gas-loaded Ti is an even more elegant one. Tritium is a β emitter with a half-life of 12.4 years. The end-point energy of betas is 18 keV. It is to be expected that β from tritium locked in Ti should excite K_α and K_β X-rays of Ti since their energies are, respectively, 4.51 keV and 4.931 keV. Using a Si (Li) detector, they found both K_α and K_β of Ti from D_2-loaded Ti samples.

I would like to make some comments on the results quoted above. There has been considerable criticism of the excess enthalpy data reported by Fleischmann *et al.* Some of the criticism seems to be

Table 1 *Production of tritium during electrolysis of D_2O*

Cathode	Electrolyte	Volume of solution (ml)	Tritium levels (Bq/ml) initial	final (10^3)	Tritium produced (no. of atoms) (10^{14})
Pd–Ag tube (M–R cell)	5 M NaOD in D_2O	250	2.6	55.6	80
Pd sheets	"	1000	2.0	7.0	40
Ti rod	"	135	2.0	1.8	1.3
Pd cylinder	0.1 M LiOD in D_2O	65	18.1	8.8	3.0

due to some misunderstanding of the term excess enthalpy. By excess heat the authors mean the heat generated over and above that due to the reactions (i) to (iv) and the Joule heat (= cell current × (cell voltage − 1.54)).

Fleischmann *et al.* estimated the heat produced from the nuclear reactions (v) and (vi) using their data of rate of neutron emission ($4\times10^{4}\,s^{-1}$) assuming a channel branching ratio of 1. They found the estimated heat to be much smaller than the experimentally observed heat. On the basis of excess heat observed they expect the fusion rate to be in the range of 10^{11}–10^{14}. In order to explain the tremendous disparity between the fusion rate based on neutron emission rate and that based on calorimetry, they proposed that some other nuclear reaction, which does not produce neutrons but produces only heat, must be occurring. In the light of the recent results of BARC, I feel that this disparity can be understood without invoking some hitherto unknown reaction. The argument of Fleischmann *et al.* was based on equal probability for the occurrence of neutron- and tritium-producing reactions. It is now known that the channel branching ratio for D–D fusion in metal lattice favours tritium production 10^{8} times more than neutron production. Therefore a neutron production rate of $4\times10^{4}\,s^{-1}$ (ref. 1) would mean production of 4×10^{12} atoms of tritium per second. Using this figure we calculate the heat expected to be released in the electrolysis of D_2O with a 10-cm-long Pd cylinder of 0.4 cm diameter to be 2.056 W/cm^2, which is approximately 50% greater than the 1.39 W/cm^2 reported by Fleischmann *et al.* This discrepancy is very much smaller than that arrived at on the basis of unity channel branching ratio, and may be explained in terms of heat taken away by the gases, fluctuation in rate of fusion reaction, etc. An excess heat of 1.39 W/cm^2 would be accounted for by production of 2×10^{12} atoms of tritium per second. Thus the tremendous disparity in the fusion rates estimated from the heat generated and the neutron flux observed can be resolved by this explanation without invoking a hitherto unknown nuclear reaction or proposing the following reaction[3]:

$$D+D\rightarrow {}^{4}He+\text{energy absorbed by the lattice.} \quad (8)$$

This reaction is far-fetched as the nuclear reaction times are several orders of magnitude smaller than the time taken for the lattice to absorb the energy[6]. If this explanation is accepted one has to find an explanation for the estimate of 1–$2\times10^{4}\,s^{-1}$ (for tritium production) arrived at by Fleischmann *et al.* from the measured tritium accumulation and the D/T separation factor. In other words, there is a discrepancy between the BARC results and those of Fleischmann *et al.* in respect of channel branching ratio. The former place it at $1:10^{8}$ whereas the latter put it at 1 : 1.

Most of the efforts of research groups appear to have been in verifying the calorimetry and neutron production rate. Confirmation of tritium production has received far less attention, and identification of He in the electrode, the least. Besides those mentioned above Storms (LANL) reported a 100-fold tritium enhancement[9] and Appleby found a million-fold tritium enhancement in 10 h[6]. It could perhaps be more meaningful to look at rate of tritium production.

Comparison of the reported results has become difficult essentially because the papers do not provide data on electrode size, time of electrolysis, number of coulombs passed, etc. For instance neutron production per unit volume of electrode can only be calculated for the results of Fleischmann *et al.* and those of Jones *et al.* The former yield 3.19×10^{4} neutrons $s^{-1}\,cm^{-3}$ while the latter give 6.15×10^{-1} neutrons $s^{-1}\,cm^{-3}$. From other reports[3,7] such data could not be calculated owing to lack of details.

To understand cold fusion, solid-state effects have been considered[8,10–13]. The possibility of increasing the penetrability of the Coulomb barrier by increasing the effective mass of electrons has been discussed. An effective mass of 6.2 would be able to account for the rate based on the neutron emission rate of Fleischmann *et al.*, and an effective mass of 11.4 would account for the rate derived from the heat data of these authors[11]. However, such a solid-state effect is not considered feasible since the effective mass effect is meaningful only for electronic motion over lengths greater than that of interatomic spacing and is irrelevant for movement over the much smaller distances involved in fusion reactions. Taking a cue from the observation that neutrons are emitted by an LiD crystal when it is fractured[14] and the suggestion[15] that cracks may be the site of fusion reactions, Kumar[13] suggests that a propagating crack in embrittled Pd or Ti may result in acceleration of deuterons to kinetic energy $\sim10^{2}$–10^{3} eV above the equipartition value, and that these deuterons may be responsible for fusion.

Iyengar[7] suggests that the very high probability for the tritium branch in cold D–D fusion reactions is indicative of transfer of neutrons across the potential barrier as suggested by Oppenheimer and

Phillips[16] and elaborated recently by Rand McNally[17]. To account for the deuteron-induced radioactivity*[18] Oppenheimer and Phillips[16] gave the following explanation. Reactions of this type can occur without requiring the penetration of a charged particle through the nuclear Coulomb barrier. When a deuteron is projected against a nucleus, the proton is held back by the Coulomb field. The neutron, which is rather weakly bound, is immune to the action of the electrostatic field, and its probability distribution can overlap the centre of the nucleus. This distribution is in fact distorted by the forces involved in the deceleration of the neutron, in such a way as to increase its density on the side towards the nucleus. Thus the neutron, which is loosely bound (binding energy ~2 MeV) in the D nucleus, is transferred to the target nucleus.

In the light of these suggestions we may propose the following hypothetical picture for the cold D–D fusion. When a metal like Pd or Ti is loaded with D_2, crack formation may occur owing to volume change. Propagation of such a crack could accelerate deuterons. (The cracking sound reported by Santhanam *et al.*[4] is indicative of such crack propagation.) These deuterons may bombard PdD_2/D held by Pd or Ti, leading to neutron capture and formation of tritium with the release of protons and energy. The released proton might transfer its energy to some other deuteron and thereby start a chain reaction, which would be terminated when a substantial portion of D in the crack tip is transmuted. The reaction would start again when another crack forms. This picture qualitatively explains the occurrence of fusion reactions in bursts and also the random distribution of reaction sites. The statistical nature of nucleation and growth of cracks and their occurrence at any point in the bulk may explain the volume effect noticed by Fleischmann *et al.* as well as the low reproducibility. This picture however fails to explain emission of neutrons. These probably form from a few of the high-energy deuterons generated by a growing crack.

I would like to point out another possibility. The deuterons accelerated by a propagating crack may hit a Pd/Ti nucleus instead of a deuterium nucleus and may transmute Pd/Ti. Such a reaction can be expected to be exothermic by analogy with other transmutations brought about by deuterium bombardment. Since the variation of transmutation yield with atomic number of target is rather small, such a reaction might be possible with nuclei heavier than copper[18]. If this type of reaction occurs, then we would have generation of heat without production of He or tritium. This possibility is worth examining.

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*When nuclei such as Na, Al, Si and Cu are bombarded with deuterons (energy 0–3.6 MeV), they capture a neutron and get transmuted into an isotope with mass one unit higher and exhibit radioactivity.

OPINION

JAPANESE INROADS INTO EUROPEAN INDUSTRY

One of the problems—a perennial one—discussed at various levels just after our independence by scientists, economists and industrialists in the country was whether foreign companies should be encouraged to start factories in India. The early policy in this country was largely against the idea. Now we follow more 'liberal policies' but the effects of these on the economy and industry are still to be assessed. One therefore reads with amusement the book *Trojan Horse*: *The Ultimate Japanese Challenge to Western Industry* by B. G. James (Mercury Business Books), which has been reviewed in many journals that deal with science, industry and finance. We quote below many sentences from this book, which are so similar to the words of the Cassandras who warned Indian planners.

'The vast majority of recent Japanese plants in Europe deserve the title assembly plants rather than factories—some are barely packaging plants to enable goods whose value is created elsewhere to be permitted to step around import controls.'

'Plants subsidized with Western tax-payers' money using Japanese-made equipment to assemble components created, designed and developed in Japan using low-skilled, low-cost Western labour make no value-added contribution to the West.'

'Such operations degrade a host nation's industry in two ways. Firstly the products are likely to be cheaper than those existing in integrated factories, so they tend to drive indigenous competitors out of business. Further the low level of skill that such plants require erodes the entire work force of Europe.'

'The Japanese-owned factories which are springing up in Europe erode the Continent's industrial base and represent the final phase in Japan's domination of world industry.'

'The arrival of multinationals three decades ago raised similar fears—but unlike the true multinationals most Japanese companies run their overseas subsidiaries as direct appendages, producing the same product all over the world.'

'Although Japanese consumer electronics firms hold 20 per cent of the colour television market and 90 per cent of the VCR market in Europe and have local assembly plants within EEC since 1975 they employ less than 50 individuals in development and none whatsoever in research.'

The bottom line of the book: 'The starting of Japanese factories in Europe and the US are not just "Trojan horses" but are time bombs ticking away at the heart of the West's industrial competitiveness.'

The storm that has been generated in the minds of many thinking people in Europe when Japanese industries are opening factories there is reminiscent of the debates that went on in this country when foreign industries were to open factories here.

We welcome views from our readers (industrialists, technologists and scientists) on whether the fears expressed in regard to the starting of Japanese factories in Europe are applicable to the case of foreign industrial companies (not necessarily Japanese) starting factories in India.

S. R.

CURRENT SCIENCE—50 YEARS AGO

THE SCOPE AND LIMITATIONS OF PHYSICAL ANTHROPOLOGY*†

PHYSICAL anthropology is the study of Man as an animal. As the physical nature of Man underlies all his cultural activities, physical anthropology is the most fundamental among the subdivisions of anthropological science. As contributions to the knowledge of Man are made by numerous departments of science, periodical evaluation and review of specialist data are of importance to keep up the coherence of physical anthropology and also to maintain intelligent contact between representatives of the different branches of anthropology.

ZOOLOGICAL POSITION OF MAN

Recent studies in comparative anatomy, embryology and physiology substantiate in general the orthodox view of anthropologists that a common ancestral stock has given rise to Man and the anthropoid apes, but this view requires to be modified in several points of detail on account of the factor of convergence that complicates human phylogenetic problems. "Resemblance is no proof of relationship", but may be due to parallelism in evolution. For example, the simian features of the extinct lemurs of Madagascar have to be attributed to parallelism, and contrary to the common accepted classification, Lemuroidea cannot be regarded as having given rise to the higher primates, as, in early geological times, they showed specializations which were avoided by the latter. If the palaeontological evidence that irreversibility is a general feature of evolutionary development be accepted, it may be inferred that the ancestral stock from which Man came did not have limbs that were specialized for arboreal life. This will lead us to the conclusion that the man-like characters of the gorilla are parallel developments. It however remains true that Man has a simian ancestry, and G. G. Simpson's superfamily, *Hominoidea*, which includes both Man and anthropoid apes is justified. Comparative physiology of *Hominoidea* is also complicated by the effects of parallel developments. Similar blood groups have, according to Zuckerman, arisen independently in Man and anthropoid apes.

PALAEONTOLOGICAL EVIDENCE OF HUMAN ORIGIN

The solution of most of our problems of human phylogeny will, in future, depend on fossil records as they turn up. Such fossil evidences as are now available are meagre, and have been made much of. Some of the primitive Miocene anthropoids of the old world, particularly *Dryopithecus*, show striking resemblance in their dentition to Man. The splitting up of the *Hominoidea* into several genera appears to have happened early in Miocene times. No Pliocene Man is known to us in spite of the evidence offered by stone tools referred to that age. The earliest *Hominidae* discovered are *Pithecanthropus* and *Sinanthropus*. Taking into consideration the relatively greater variability of Man, it appears that anthropologists have exaggerated the points of difference between *Pithecanthropus* and *Sinanthropus* and made two genera of them instead of one. While the skull, brain and teeth in *Pithecanthropus*, retained primitive simian characters, the limb bones were like those of *Homo*. This is of significance in showing that the differences in limb structure between Man and anthropoid apes are very old, and that the divergence between the two groups must have taken place at a relatively remote period.

Neanderthal Man of later Mousterian date was more specialized than modern Man, who, it seems certain, was derived, not from these extreme Neanderthals, but from more generalized types of earlier date.

The study of endocranial casts is useful but has its own pitfalls. The convolution patterns in Man and anthropoid apes are not correctly impressed on the bones as in the lower animals. Too much emphasis has been laid in the past on the 'simian sulcus' in the study of endocranial casts of fossil *Hominids*, but Elliot Smith has shown how misleading this 'simian sulcus' can be. According to him, some modern human brains occasionally develop a sulcus which is easily mistaken for the simian sulcus.

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*Summary of Address by Prof. W. E. Le Gros Clark, F.R.S., President, Section H—Anthropology, British Association for the Advancement of Science, Dundee, 1939.

†Published in *Curr. Sci.*, 1939, **8**, 487.

In the study of individual skeletons there are considerable difficulties due to our inability to eliminate variations due to differences in habits, diet, etc. Even the determination of sex offers difficulties when only skulls are available for study.

PHYSICAL ANTHROPOLOGY OF RACE

There is considerable overlap in racial characters even among primary races owing to the 'reticulate evolution' of Man. Many of the racial characters now adopted are themselves susceptible to environmental influences, which may obscure fundamental similarities. Blood groups are more reliable, but there seems to be no correlation between them and body types. The determination of racial characters of pre-historic peoples from a study of their skeletons is again of uncertain value. The Grimaldi skulls of Europe, for example, were regarded as Negroid, but Elliot Smith was of opinion that they were merely variants of the Mediterranean race. R. A. Fisher has also shown the greater advantages of the study of the living over that of skeletal material.

THE FUTURE OF PHYSICAL ANTHROPOLOGY

With the handicaps inherent in the material, and with the existing technique, it is doubtful if sensational progress will be made in physical anthropology. But biometry still holds the key to the understanding of the composition of geographical groups of Man. Physical anthropology will have to become more of a field science and study Man as he is today, attacking such problems as the relation of nutrition to physique, effects on physical types of change of environment, the phenomenon of twinning, the relation of bodily types to mental traits, etc. Human genetics will have to be studied by the anthropological method. Various formulae have been devised for assessing the nutritional status, but anthropologists will have to determine what the normal physical type is for a given population.

A. Aiyappan

THE COCONUT PALM BEETLE

This familiar coconut pest commonly known as the rhinoceros beetle is one of the most discouraging and distracting features in the cultivation of the coconut tree in many parts of South India where it is more responsible than any other single factor for causing a serious set-back to the young growing tree, leading in the case of neglect to a complete destruction of the tree. It is probably one of the pests regarding which requests for suitable remedies are received most frequently. A considerable amount of study both in respect of its life-history and of remedial methods has been made but it is nevertheless a fact that many lacunae exist in the former while as regards remedies no satisfactory ones are yet known. On both of these aspects and especially in connection with the life-history of the pest a careful study extending over a long period has been made, the results of which are now published (M. C. Cherian and K. P. Anantanarayanan, *Indian J. Agric. Sci.*, 9, Pt. III). The duration of the egg period, the larval and pupal periods are all subject to considerable variation and are found to be 9 to 17 days, 100 to 180 days, and 24 to 62 days respectively, the period from egg to adult varying from 129 to 232 days. The adults themselves were found to live for periods up to a maximum of 293 days. It is also brought out that the beetles are active throughout the year although during certain months of the year, viz. March and April, the pest is most active; elsewhere too this is the same experience, the peak of the damage being soon after the first rains begin. Work on remedial methods which of course are more important from a practical view-point has not led to any helpful recommendations; a trial has been made of various baits, none of which was found of any use. We have noted however that a mash made up of a little groundnut oil cake with cow-dung proved remarkably effective as a bait. We find no reference to the spraying with Bordeaux Mixture which was tried as a good repellent in certain Mysore trials. Various other devices which are probably mere 'nostrums' but which may have something in them also deserve to be tested out especially in a thorough going investigation of this kind. The growing alongside coconut plants of plantains and of *Euphorbia tirucalli*, the use of common salt, nux vomica leaves and fruit, the oil cakes of some species of Hydnocarpus are mentioned as repellents of this category—inquiries may bring out more—and these deserve a trial. The familiar skewering out with an iron rod with a barbed end apparently holds the field, in the trials reported.

A. K. Y.

ANNOUNCEMENT

SCIENTIFIC MEETING ON TROPICAL DISEASE RESEARCH

The World Health Organization—Tropical Diseases Research and Rockefeller Foundation First Annual Scientific Meeting will be held at ASTRA Research Centre India from 3 to 8 December 1989. Scientists from all over the world who are receiving research support from WHO/RF will be attending this meeting. Although this is a closed meeting, arrangements have been made for closed-circuit television viewing. Those who are interested in coming to this meeting may write or contact:

WHO/RF Meeting Committee
ASTRA Research Centre India
18th Cross, Malleswaram
Bangalore 560 003

Phone: 340373 Telex: 845 2011 ARCI IN
Fax: 340 449.

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ARTICLES

CRYSTAL STRUCTURE AND FIVE-FOLD DIFFRACTION SYMMETRY OF THE ALUMINIUM–MANGANESE ICOSAHEDRAL PHASE: A REAPPRAISAL

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ABSTRACT

A model is developed for the crystallization of icosahedral phases in Al–Mn, Al–Mn–Si and allied systems, starting from the icosahedral clusters and ordered pentagonal chains of atoms postulated a few decades ago to exist in alloy melts. It is shown that different traditional crystal structures can emerge through the joining together in regular fashion of the pentagonal chains of atoms in the special conditions of rapid solidification. The primary unit for the building of such structures is the primitive *orthorhombic* subcell that forms by joining 13-atom icosahedra in three perpendicular directions, through *vertex* connection along the first axis, *edge* connection along the second axis and *triangular ledge* connection along the third axis. A family of orthorhombic unit cells can be visualized to develop from this model through *complex* ordering involving two or more species of atoms and consequent *multiplication* of the subcell parameters. The orthorhombic unit cell dimensions are arrived at on the basis of this model for the $Al_{80}Mn_{20}$ icosahedral phase and it is shown that the position of X-ray and electron reflections can be satisfactorily explained by this unit cell.

It is also shown that this model for growth of icosahedral phases permits emergence of *orientation variants* of the same crystal 72° to each other as well as random changes in orientation and growth directions on a submicroscopic scale. Each grain or 'crystal' of such icosahedral phases thus turns out to be a complex mosaic made up of five sets of irregular-shaped microcrystallites of nanometre dimensions of a traditional orthorhombic phase, each fully or partly coherent with and oriented 72° to its neighbours. Such a state of affairs is shown to account for the unusual microstructural and some other features of these exotic phases.

INTRODUCTION

A number of interesting models have been proposed[1,2] since 1985 to explain the atomic arrangements in intermetallic phases obtained by rapid solidification of mostly aluminium-based alloys and displaying five-fold symmetry in their X-ray and electron diffraction patterns. All these models generally assume quasiperiodicity of atomic arrangements and deal with non-traditional lattices arrived at by novel approaches like three-dimensional Penrose tiling, inflation–deflation operations, generalized multi-grid methods and strip projection and cut projection from high-dimensional periodic lattices. The location of atoms in the rigid geometrical frameworks thus proposed, i.e. the so-called decoration of the quasilattices, has however presented some difficulties like unacceptable density or peculiar interatomic distances, and no model has been able thus far to explain satisfactorily all observed features of these exotic phases.

As documented earlier[3], a few attempts have also been made[4-7] since 1985 to explain the unusual diffraction effects from these so-called icosahedral phases on the basis of interpenetrating multiple microtwins of traditional crystals. However, the unit cells proposed subsequently for such phases, and particularly for the $Al_{80}Mn_{20}$ icosahedral phase, by Pauling[8], the present author[9], and Khan and Wibbeke[10] have not been supported by convincing arguments or models for their formation, nor accompanied by any detailed description of the actual number and the exact location of the concerned atoms in them to explain satisfactorily *both* positions and intensities of the reflections in diffraction patterns. The case for the presence of twins or orientation variants on a submicroscopic scale in such phases has been reopened most recently in a rather spectacular way by the findings of Vecchio and Williams[11,12]. Studying wedge-like specimens of the Al–Li–Cu icosahedral phase, these workers have not only brought out the existence of numerous nanometre-scale microcrystallites in micron-size grains, but also demonstrated the absence of five-fold symmetry in very thin sections of their specially prepared specimens. In fact convergent beam electron diffraction patterns showed evidence for *just* single crystals in the thinnest sections examined.

BASIS OF NEW MODEL

A model is developed in this paper for the crystallization of icosahedral phases in Al–Mn, Al–Mn–Si and allied systems, starting from icosahedral clusters[13] and pentagonal chains[14] of atoms in the concerned metallic melts. The existence in liquid metals of icosahedral clusters containing 13 atoms each (figure 1, a–c) and characterized by high density as well as low energy was postulated by Frank[13] four decades ago to explain the marked undercooling recorded by small drops of liquid metals. Despite their high co-ordination number such icosahedra cannot constitute nuclei for the growth of solid crystals with close-packed structures. In the case of *alloy* liquids the Frank icosahedra were postulated almost three decades ago by Hume-Rothery and Anderson[14] to join together through sharing of the vertex atoms (figure 1, d,e) when the sizes, valencies and electronegativity values of the concerned atoms differ suitably to confer stability to the resulting pentagonal chains. The emergence of peaks in eutectic distribution curves, i.e. the stabilization of the liquid state at certain compositions, in binary alloy systems could be explained on the basis of such *ordered* pentagonal chains of atoms.

As elucidated by Hume-Rothery and Anderson[14], if solute B is added to liquid metal A, mixing is encouraged whenever there is a pronounced tendency for B atoms to prefer A rather than B neighbours. Thus pentagonal chains made up of different repeating units in an AB alloy can acquire stability at certain compositions, like the following, through avoidance of B–B contacts:

I. 1A–5A–1B–5A — 1A–5A–1B–5A ———— 1/12 or 8.33 at.% B

II. 1B–5A–1B–5A ———————— 1/6 or 16.67 at.% B

III. $1\mathrm{A}-\genfrac{}{}{0pt}{}{3\mathrm{A}}{2\mathrm{B}}-1\mathrm{A}-\genfrac{}{}{0pt}{}{4\mathrm{A}}{1\mathrm{B}}\ 1\mathrm{A}-\genfrac{}{}{0pt}{}{3\mathrm{A}}{2\mathrm{B}}-1\mathrm{A}-\genfrac{}{}{0pt}{}{4\mathrm{A}}{1\mathrm{B}}$ ———— 1/4 or 25.00 at.% B.

The present model for icosahedral phase formation starts with the idea that different crystal structures can in fact emerge through the joining together of such pentagonal chains in special circumstances, particularly when appreciable or drastic kinetic undercooling of the alloy melt is brought about by techniques like rapid solidification.

Although novel, the concept advanced here of icosahedra and/or icosahedral chains *themselves constituting nuclei* for the growth of crystals from metallic melts is by no means a startling one. Apart from α-rhombohedral (figure 2a) boron, whose structure is built up of regular icosahedra of atoms in slightly deformed cubic close packing, its α-tetragonal compounds $B_{50}C_2$ and $B_{50}N_2$ (figure 2b) are known to be made up of slightly distorted icosahedra of B atoms linked by C and N atoms respectively[15]. In regard to such structures it has also been recognized that the generation of an infinite three-dimensional lattice with the *perfect* five-fold symmetry of ideal icosahedra is impossible in practice and one should be prepared for distortions, translations, voids, chemical inhomogeneities, lattice parameter variations, etc. in them. Even in alloy systems the growth of complex cubic phases made up of clusters of atoms formed around icosahedra has been known for some time[16]. The cubic phases with the composition $A_{12}B$ in Al–W, Al–Cr and Al–Mn systems seem to be made up of thirteen-atom icosahedra (figure 2c) aligned without any atom sharing to generate their 26-atom body-centred cubic (b.c.c.) unit cells[17].

The occurrence of structural units with icosahedral symmetry, even if they are distorted more often than not, in a large number of complex intermetallic phases has recently been highlighted in a review[18].

It is interesting to record here that the very first model suggested for the structure of the i-Al–Mn phase[19] was based on icosahedra joined by their edges, although not regularly but *randomly* and without any long-range order. This approach was further developed by Stephens and Goldman[20] into the so-called icosahedral glass model with vertex-sharing icosahedra packed randomly while maintaining bond-orientational order. These could explain the sharp peak positions in diffraction patterns, but features like peak intensities could not be satisfactorily accounted for by them.

GROWTH OF ORTHORHOMBIC STRUCTURES

The present model envisages growth of different crystalline phases with orthorhombic or related

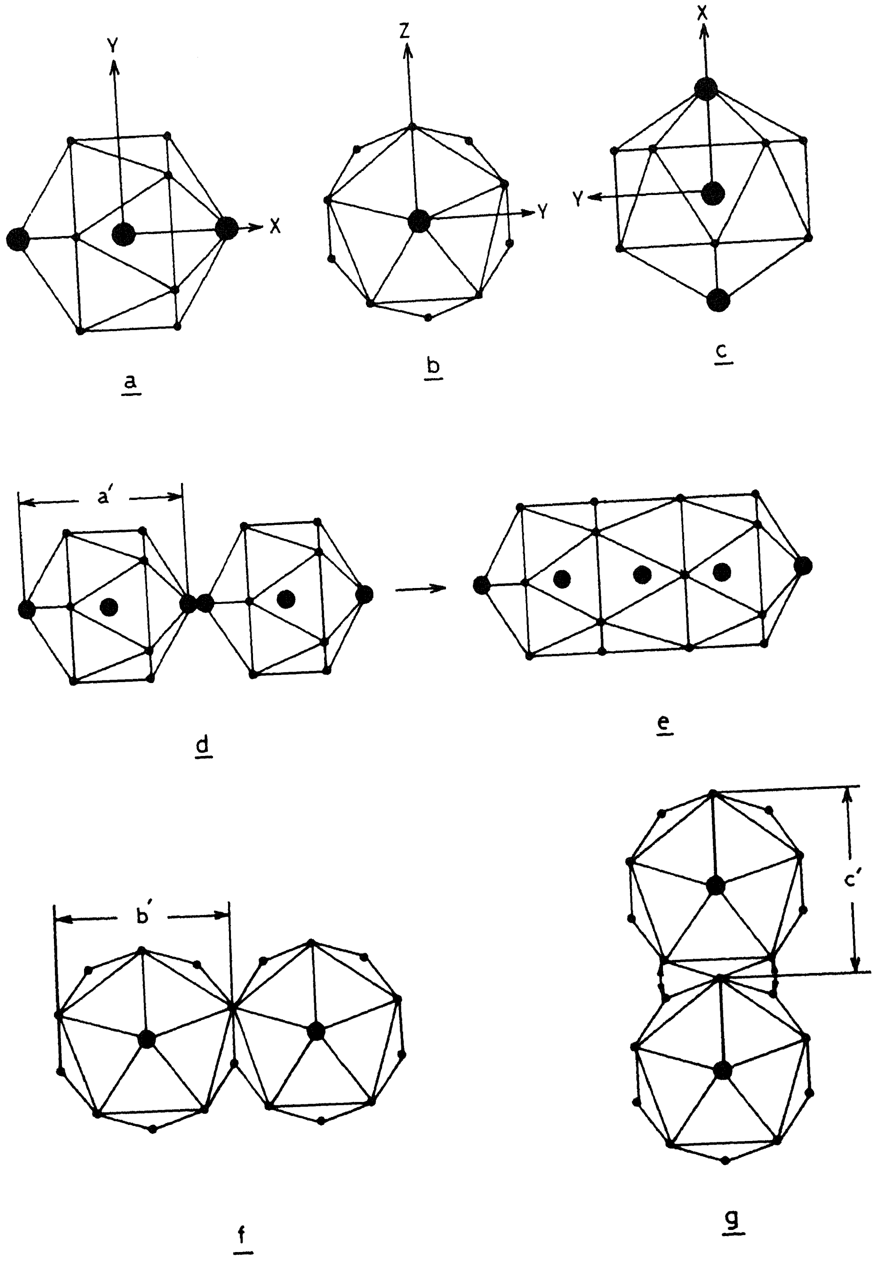


Figure 1. Three different projections (a, b and c) of an icosahedron to illustrate the emergence of a primitive orthorhombic subcell by the coming together of icosahedra through *vertex* connection along the *x*-axis, *edge* connection along the *y*-axis and *triangular ledge* connection along the *z*-axis respectively (d, f and g). Vertex connection leading to the formation of the so-called pentagonal chains (e) and arrows highlighting atom sharing in triangular ledge connection (g) are illustrative of the distortions in the icosahedra during formation of the orthorhombic crystal structure.

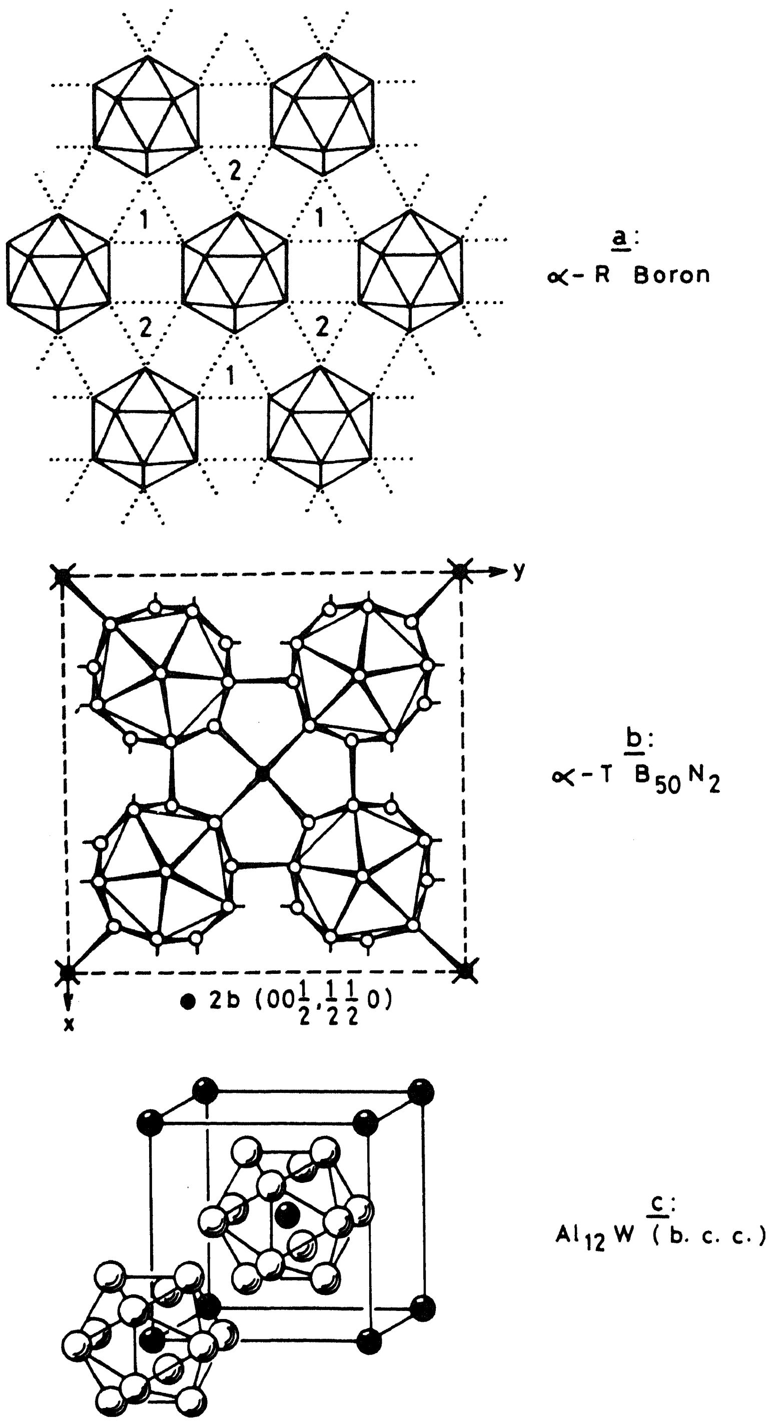
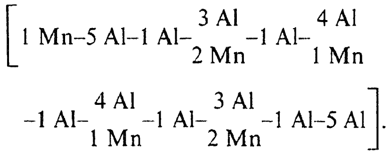


Figure 2. Crystal structures based on geometrical arrangements of the icosahedra: a, α-rhombohedral boron; b, α-tetragonal boron nitride; c, body-centred cubic $Al_{12}W$ intermetallic phase.

crystal structures through the coming together in a *regular* fashion and in all *three* directions of the pentagonal chains (figure 1, d,e) made up of vertex-sharing icosahedra in alloy melts. The primary *unit* for the building of such crystal structures is the primitive orthorhombic *subcell* that forms by joining 13-atom icosahedra (figure 1, d–g) through *vertex* connection along the x-axis, *edge* connection along the y-axis and *triangular ledge* connection along the z-axis. If the length of one of the 30 edges of the icosahedron is l, the edge lengths a', b' and c' for this subcell will work out on the basis of geometry to 1.902 l, 1.618 l and 1.701 l respectively, *provided* there is no distortion at all of the icosahedra through the proposed joining and atom sharing. In actual practice, apart from the value of l fluctuating in accordance with the *actual* occupancy by A, B, C, etc. atoms of the icosahedron, the sizes, valencies, positions, etc. of the concerned atoms and the nature of atom sharing may be expected to distort the icosahedra and modify a', b' and c' appreciably. It is not difficult, however, to visualize the growth of *several* orthorhombic unit cells, base-centred, body-centred or face-centred, through *complex ordering* involving several species of atoms, and the consequent enlargement of the cell parameters to $m{\cdot}a'$, $n{\cdot}b'$ and $o{\cdot}c'$ respectively, where m, n and o are integers. In some respects such orthorhombic structures can be viewed as a set or family of *polytypes*, the occurrence of which has been strongly suspected in the case of *decagonal* phases of Al–Mn, Al–Fe, Al–Co and related systems[21].

Considering the icosahedral phases of the Al–Mn, Al–Mn–Si and closely related alloy systems, it is possible to arrive at the values of m, n and o and the nature of the orthorhombic unit cell by a careful analysis of X-ray diffraction data, wherein the strong and medium reflections are less than 20, the weak and very weak reflections run into perhaps hundreds, and the highest observed d value as close to 1.0 nm. While Pauling[8] has proposed a cubic unit cell with $a = 2.336$ nm, the present author was able to show very good agreement[3] between observed and calculated d values of the same $Al_{80}Mn_{20}$ icosahedral phase on the basis of a smaller tetragonal unit cell with $a = 1.653$ nm and $c = 1.736$ nm. The most recent studies[22] on the stoichiometry of Al–Mn-based icosahedral phases have confirmed some previous estimates of around 20 at.% solute elements in these phases, even though the first and most-quoted paper on i-Al–Mn phases[23] had insisted on the formula Al_6Mn for this phase. Among the three types of pentagonal chains referred to earlier, a combination of II and III leads to the following repeating unit of the chain:



$$\Big[\,1\,\mathrm{Mn}\text{–}5\,\mathrm{Al}\text{–}1\,\mathrm{Al}\text{–}\begin{matrix}3\,\mathrm{Al}\\2\,\mathrm{Mn}\end{matrix}\text{–}1\,\mathrm{Al}\text{–}\begin{matrix}4\,\mathrm{Al}\\1\,\mathrm{Mn}\end{matrix}$$

$$\text{–}1\,\mathrm{Al}\text{–}\begin{matrix}4\,\mathrm{Al}\\1\,\mathrm{Mn}\end{matrix}\text{–}1\,\mathrm{Al}\text{–}\begin{matrix}3\,\mathrm{Al}\\2\,\mathrm{Mn}\end{matrix}\text{–}1\,\mathrm{Al}\text{–}5\,\mathrm{Al}\,\Big].$$

This in turn gives a value of 3 for m and the generally accepted ~20 at.% Mn for the phase with 7 Mn atoms and 29 Al atoms in the repeating unit. A value of 4 each for n and o follows from the parameters already arrived at by the author and referred to earlier. Such a large unit cell seems to be *necessary* for the complex ordering between Al and Mn atoms in the structure built around 3-unit pentagonal chains through sharing of ALL but the central atom in each icosahedron by neighbouring icosahedra. The i-Al–Mn and allied phases thus acquire base-centred (A) orthorhombic unit cells made up of 48 (3 × 4 × 4) primitive orthorhombic subcells and *perhaps* 336 (7 × 48) atoms in them. The nature of atom sharing, which is obviously complex, will decide whether the proportion between Al and Mn atoms remains the *same* i.e. 29 to 7, as in the seed pentagonal chains.

Table 1 brings out the clear picture that emerges on the basis of the model proposed and the considerations detailed above. We start with the reasonable value of 0.26 nm for the *average* Al–Mn distance, i.e. for the *average* edge length in the icosahedra

Table 1 *Orthorhombic lattice parameters in nanometres for Al–Mn icosahedral phase*

Parameter	i-$Al_{80}Mn_{20}$
a'_i	0.4946
b'_i	0.4207
c'_i	0.4424
a'	0.5096
b'	0.4137
c'	0.4344
a	1.5288
b	1.6548
c	1.7376

Parameters a'_i, b'_i and c'_i are for the ideal subcell, the edge of the icosahedron (l) being 0.26 nm; a', b' and c' are for the actual subcell; and a, b and c for the unit cell.

constituting the building block for the orthorhombic crystal structure of i-Al–Mn, i-Al–Mn–Si and related phases. Recent studies, using neutron diffraction and contrast variation effects, of the partial pair distribution functions in i-Al–Mn and allied phases have yielded[24] average Al–Mn distances of 0.255 nm for a co-ordination number of 9.4 and 0.305 nm for a co-ordination number of 2.4. Significantly, no close contacts between Mn atoms could be observed in these studies, justifying the concept of ordered pentagonal chains constituting nuclei for the growth of icosahedral phases in the present model. In table 1 a_i', b_i' and C_i' represent the *ideal* parameters for an orthorhombic subcell based on regular icosahedra with edge length 0.26 nm and with no distortion due to atom sharing . Parameters a', b' and c' are the *actual* parameters of the primitive orthorhombic subcell following distortions through occupancy by atoms of *different* sizes, valencies, etc. and also atom sharing, while a, b and c refer to the actual *lattice parameters* of the i-Al–Mn phase on the basis of the values 3, 4 and 5 arrived at for m, n and o respectively.

It is very significant that despite obvious distortions due to atom sharing, one of the axial ratios, viz. c/b, of the orthorhombic phase almost coincides with the ideal value of 1.0515 for a regular icosahedron (table 1). In fact it was the highlighting of this axial ratio by Mackay[25] in regard to the tetragonal unit cell proposed earlier[3] that led the author to think afresh on this problem and develop the present model. Another point to be highlighted here is the slight expansion in the value of a' in relation to a_i' (table 1) brought about by vertex joining in the pentagonal chain, resulting perhaps in the *equalization* of distances between central atoms and the planes of pentagons. Incidentally, it may be noted in passing that orthorhombic and rhombohedral structures are closely related to *cubic* structures and can be derived from the latter by slight distortions of the cubic unit cell.

The mechanism envisaged in the present model for the growth of icosahedral (and perhaps also decagonal) phases in general permits the emergence of orientation variants of the same crystal 72° to each other, as illustrated in figure 3. In fact, with a large number of pentagonal chains of differing lengths obviously available to serve as seeds for nucleation, not only can the grain sizes be expected to be small (as actually observed), but also random or quasiperiodic changes in orientation and growth directions on a submicroscopic scale can be envisaged. Thus *each* grain or crystal of such icosahedral phases can turn out to be a complex

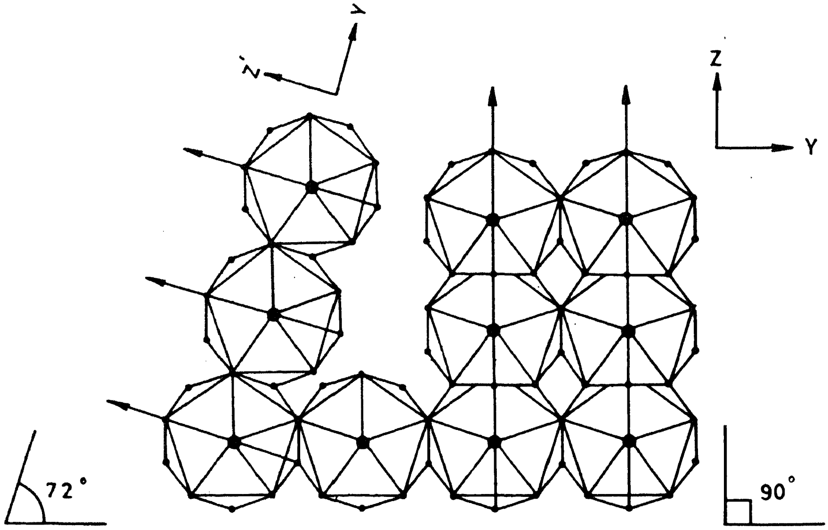


Figure 3. Illustration of a change in orientation during growth of the orthorhombic crystal from pentagonal chains of atoms. Five orientations 72° to each other can be conceived through random changes in growth direction for the nanometre-size microcrystallites constituting a grain of the icosahedral phase.

mosaic made up of five sets of irregular-shaped microcrystallites on nanometre scale of the traditional orthorhombic phase, grown around one or more parallel pentagonal chains, each fully or partly coherent with and oriented 72° to its neighbours. Such a state of affairs has in fact been noted by many workers[4, 11, 26–28] in regard to different icosahedral phases.

ANALYSIS OF DIFFRACTION DATA

Tables 2 and 3 present results of analysis of the X-ray diffraction data obtained for the i-Al–Mn phase with synchrotron radiation[29]. Table 2 shows how most of the strong and medium reflections from i-$Al_{80}Mn_{20}$ can be most satisfactorily indexed on the basis of *both* the subcell and the actual base-centred (A) orthorhombic unit cell, highlighting the fact that a few medium-intensity and most of the large number of weak and very weak reflections are due to the *complex ordering* between the concerned species of atoms during crystallization. Table 3 shows that all 48 reflections recorded thus far and referred to in many publications on i-Al–Mn can again be very satisfactorily accounted for on the basis of the proposed unit cell. It is interesting to note that most of the weak reflections at higher Bragg angles can also be indexed on the basis of the concerned subcell described in table 1. Apart from the excellent agreement between calculation and observation and the strict adherence to extinction rules applicable to such structures (table 3), the most encouraging aspect of the analysis here is that the strong and medium reflections in table 1 display indices that one would expect from a cursory examination of the atom positions in the subcell (figure 1, d, f, g).

Table 2 *Calculated and observed interplanar spacings (d_{cal} and d_{obs}) in nanometres with observed relative intensities (I, rounded to multiples of 5) for the strong and medium X-ray reflections from the i-$Al_{80}Mn_{20}$ phase, which get indexed on the basis of both the subcell (h′ k′ l′) and the unit cell (h k l) described in table 1*

			i-$Al_{80}Mn_{20}$		
No.	h′ k′ l′	h k l	d_{cal}	d_{obs}	I
1	002	008	0.2172	0.2172	100
2	020	080	0.2069	0.2068	80
3	022	088	0.1497	0.1497	10
4	400	12.00	0.1274	0.1275	20
5	402	12.08	0.1099	0.1101	10
6	004	00.16	0 1086	0.1085	10
7	420	12.80	0.1085		
8	040	0.16.0	0.1034	0.1033	5

Table 3 *Calculated and observed interplanar spacings (d_{cal} and d_{obs}) in nanometres with observed intensities (I) for all, i.e., strong, medium and weak, observed X-ray reflections from i-$Al_{80}Mn_{20}$ phase*

No.	h k l	d_{cal}	d_{obs}	I
1	013	0.5467	0.542	<1
2	031	0.5257		
3	024	0.3846	0.385	22
4	242	0.3356	0.335	8
5	333	0.3144	0.314	<1
6	404	0.2870	0.286	2
7	026	0.2733	0.274	<1
8	306	0.2518	0.252	3
9	046	0.2373	0.238	<1
10	622	0.2345	Al	—
11	$008^{\bullet}$	0.2172	0.217	100
12	$640^{\bullet}$	0.2170		
13	337	0.2069	0.207	78
14	$080^{\bullet}$	0.2069		
15	$644^{\bullet}$	0.1941	0.194	1
16	175	0.1939		
17	606	0.1913	0.191	<1
18	800	0.1911		
19	375	0.1825	0.183	<1
20	480	0.1819	0.182	<1
21	735	0.1753	0.175	2
22	093	0.1753		
23	804	0.1749		
24	664	0.1719	0.172	<1
25	448	0.1718		
26	557	0.1665	0.166	<1
27	268	0.1665		
28	$680^{\bullet}$	0.1606	0.160	1
29	806	0.1595		
30	$088^{\bullet}$	0.1498	0.150	11
31	577	0.1494		
32	775	0.1457	0.145	3
33	$00.12^{\bullet}$	0.1448		
34	$0.12.0^{\bullet}$	0.1379	0.137	<1
35	$04.12^{\bullet}$	0.1367		
36	884	0.1336	0.134	<1
37	$12.00^{\bullet}$	0.1274	0.127	20
38	$60.12^{\bullet}$	0.1259	0.126	<1
39	$984^{\bullet}$	0.1257		
40	$12.04^{\bullet}$	0.1223	Al	—
41	$6.12.0^{\bullet}$	0.1213	0.121	1
42	80.12	0.1154	0.114	1
43	$90.12^{\bullet}$	0.1102	0.110	6
44	$12.08^{\bullet}$	0.1099		
45	$00.16^{\bullet}$	0.1086	0.109	7
46	$12.80^{\bullet}$	0.1085		
47	$68.12^{\bullet}$	0.1075	0.108	3
48	$0.16.0^{\bullet}$	0.1034	0.103	2

Al, Possible overlap of Al reflections. $hkl^{\bullet}$, such *hkl* values are also indexable on the basis of the subcell. Subcell and unit cell parameters as in table 1.

Figure 4 brings out, through computer simulation of the five possible orientation variants of the orthorhombic phases under discussion, the emergence of the familiar electron diffraction patterns with five-fold symmetry, wherein *most* of the spots are due to dynamic double diffraction, as first suggested by Ball

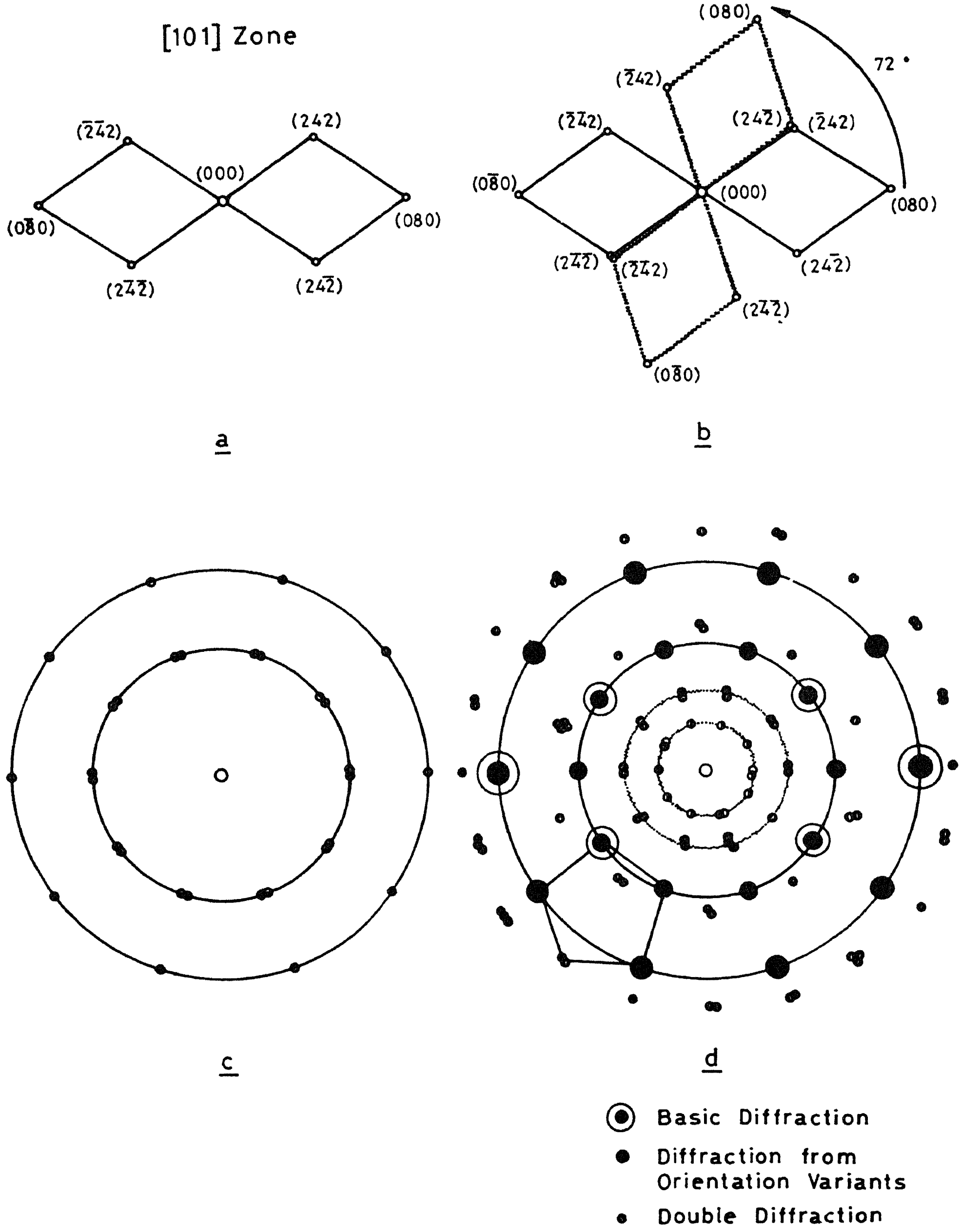


Figure 4. Computer-simulated electron diffraction pattern (d) consisting of basic diffraction, orientation variant diffraction and dynamic double diffraction spots for i-Al–Mn phase. The starting point here (a) is the single crystal pattern from the [101] zone. Two orientation variants and all five orientation variants lead to patterns b and c respectively, ignoring double diffraction.

and Lloyd[4] with regard to the i-Al–Li–Cu phase. The starting point in this rather striking demonstration is the single crystal diffraction pattern (figure 4a), as computer-simulated for the [101] zone of the orthorhombic i-Al–Mn phase. Starting with just *two* families of reflections, viz. {080} and {242}, the highly impressive and by now famous five-fold pattern emerges (figure 4d), with *dozens* of reflections, some due to orientation variants but *most* due to double diffraction. Because the interplanar distances of the permitted 242-reflection and the forbidden 050-reflection, viz. 0.334 and 0.331 nm respectively, are *very close* to each other, the so-called Fibonacci sequence of reflections, viz. (020), (030), (050), (080), and (0.13.0), appears fortuitously because of double diffraction, conjuring thereby illusory visions of quasiperiodicity in atomic arrangements for these phases.

Further implications of the present model and the explanation for many other observations related to icosahedral and decagonal phases will be discussed elsewhere.

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FRACTIONAL VOTING SYSTEM: A VOTING SYSTEM TO CIRCUMVENT ARROW'S PARADOX

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ABSTRACT

Making use of information theory it is shown that fractional voting system (FVS) can be used to circumvent Arrow's Paradox. A crucial fact about FVS is that the input to the voting system is the preference distribution of the voters instead of the usual preference orders. In the FVS it is possible to associate a preference distribution for the society as a whole and it is unique if the axioms of unanimity and independence are to be satisfied. An interesting fact is that the same unique distribution results when injustice, as defined here, is minimized.

INTRODUCTION

In 1951 Arrow showed that if the input to a voting system is the preference order of each voter for the candidates, then there is no reasonable way to assign a *preference order* appropriate for the entire society[1-3]. It is shown here that the situation alters drastically if the input to the voting system is changed to the *preference distribution* of each voter and it becomes not only possible but also natural to associate a unique preference distribution for the body of voters. The voting system proposed here is called the fractional voting system (FVS) and it is further demonstrated that information theory is an efficient tool to facilitate poll analysis.

In FVS each voter has at his disposal not just one vote but a number of votes. In figure 1, N_j is the number of votes available to voter b_j.

$$\begin{array}{c c c}
 & \begin{array}{ccccc} b_1 & b_2 & b_3 & \dots & b_n \end{array} & \\
\begin{array}{c} a_1 \\ a_2 \\ \vdots \\ a_m \end{array} & \left[\begin{array}{ccccc} k_{11} & k_{12} & k_{13} & \dots & k_{1n} \\ k_{21} & k_{22} & k_{23} & \dots & k_{2n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ k_{m1} & k_{m2} & k_{m3} & \dots & k_{mn} \end{array}\right] & \begin{array}{c} M_1 \\ M_2 \\ \vdots \\ M_m \end{array} \\
 & \begin{array}{ccccc} N_1 & N_2 & N_3 & \dots & N_n \end{array} & N
\end{array}$$

Figure 1. Matrix $K=[k_{ij}]$.

This voter can distribute his N_j votes to the different candidates in any manner he pleases. The matrix $K=[k_{ij}]$ is called a *voting pattern*. The integer k_{ij} is the number of votes given by the voter b_j to the candidate a_i. Thus

$$\sum_{i=1}^{m} k_{ij}=N_j.$$

The total number of votes collected by the candidate a_i is given by

$$\sum_{j=1}^{n} k_{ij}=M_i.$$

Thus

$$\sum_{i=1}^{m} M_i=N$$

and $\sum_{j=1}^{n} N_j=N.$

After collecting the voting pattern as usual, the candidate who collects the maximum number of votes is declared as the winner by FVS.

$$\begin{array}{c c c}
 & \begin{array}{ccccc} b_1 & b_2 & b_3 & \dots & b_n \end{array} & \\
\begin{array}{c} a_1 \\ a_2 \\ \vdots \\ a_m \end{array} & \left[\begin{array}{ccccc} p_{11} & p_{12} & p_{13} & \dots & p_{1n} \\ p_{21} & p_{22} & p_{23} & \dots & p_{2n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ p_{m1} & p_{m2} & p_{m3} & \dots & p_{mn} \end{array}\right] & \begin{array}{c} q_1 \\ q_2 \\ \vdots \\ q_m \end{array} \\
 & \begin{array}{ccccc} r_1 & r_2 & r_3 & \dots & r_n \end{array} &
\end{array}$$

Figure 2. Matrix $P=[p_{ij}]$.

In figure 2,

$$p_{ij}=k_{ij}/N, \quad q_i=M_i/N, \quad r_j=N_j/N.$$

A matrix with non-negative elements which add up to one is called a *distribution*. An element of such a matrix is called a *mass*. The matrix $P=[p_{ij}]$ gives the preference distribution of the electorate for the candidates. The matrix $[r_j]$ gives the *prominence* *distribution* of the voters within the society. If

$$q_{ij} = p_{ij}/r_j$$

then each column of $Q = [q_{ij}]$ gives the preference distribution of an individual voter for the candidates. If

$$r_{ij} = p_{ij}/q_i$$

then each row of $R = [r_{ij}]$ gives the *affinity distribution* of a candidate for the voters.

We can now state the difference between the usual voting systems (UVS) and FVS. In UVS the input to the voting system is the *preference order* of a voter and each voter has a single vote[1-3]. In FVS the input is the *preference distribution* of a voter for the candidates and the *prominence distribution* of the voters within the society. Arrow[1] has shown that in the case of UVS, it is impossible for the society to have a reasonable preference order for the candidates if the axioms of unanimity and independence are to be satisfied. The main purpose of this paper is to show that in the case of FVS, it is possible for the society to have a preference distribution for the candidates satisfying both unanimity and independence as defined here. Further, it is shown that this distribution is unique.

INFORMATION THEORY AND PSEPHOLOGY

With FVS it is possible to carry out a thorough analysis of the poll. For the purpose we take some concepts from information theory and make up some definitions. All logarithms mentioned here are to the base 2. In the following A represents the candidates and B represents voters.

Definitions

1. *Voter hesitance*

$$H_j(A) = -\sum_{i=1}^{m} q_{ij}\log q_{ij}.$$

2. *Voter preference*

$$I_j(A) = \log m - H_j(A).$$

3. *Conditional hesitance*

$$H(A\bar{B}) = \sum_{j=1}^{n} r_j H_j(A).$$

4. *Conditional preference*

$$I(A\bar{B}) = \log m - H(A\bar{B}).$$

5. *Panel homogeneity*

$$H(A) = -\sum_{i=1}^{m} q_i \log q_i.$$

6. *Panel heterogeneity*

$$I(A) = \log m - H(A).$$

7. *Clan uniformity*

$$H_i(B) = -\sum_{j=1}^{n} r_{ij}\log r_{ij}.$$

8. *Clan affinity*

$$I_i(B) = \log n - H_i(B).$$

9. *Conditional uniformity*

$$H(\bar{A}B) = \sum_{i=1}^{m} q_i H_i(B).$$

10. *Conditional affinity*

$$I(\bar{A}B) = \log n - H(A\bar{B}).$$

11. *Electorate homogeneity*

$$H(B) = -\sum_{j=1}^{n} r_j \log r_j.$$

12. *Electorate heterogeneity*

$$I(B) = \log n - H(B).$$

13. *Societal homogeneity*

$$\begin{aligned} H(A+B) &= \sum_{i=1}^{m}\sum_{j=1}^{n} p_{ij}\log p_{ij} \\ &= H(A) + H(\bar{A}B) \\ &= H(A\bar{B}) + H(B). \end{aligned}$$

14. *Societal heterogeneity*

$$\begin{aligned} I(A+B) &= \log mn - H(A+B) \\ &= I(A) + I(\bar{A}B) \\ &= I(A\bar{B}) + I(B). \end{aligned}$$

15. *Election campaign*

$$\begin{aligned} H(AB) &= H(A) + H(B) - H(A+B) \\ &= H(A) - H(A\bar{B}) \\ &= -H(\bar{A}B) + H(B). \end{aligned}$$

16. *Election propaganda*

$$\begin{aligned} I(AB) &= -H(AB) \\ &= I(A)+I(B)-I(A+B) \\ &= I(A)-I(A\bar{B}) \\ &= -I(\bar{A}B)+I(B). \end{aligned}$$

17. *Popularity of a candidate*

$$P_i = \log mq_i.$$

18. a_i is a *popular candidate* if $P_i \geq 0$.
19. a_i is an *eminent candidate* if he is the only popular candidate.
20. a_i is a *favourite candidate* if $P_i \geq I(A)$.
21. a_i is an *outstanding candidate* if he is the only favourite candidate.
22. a_i is a *charismatic candidate* if he collects all the votes without exception.
23. *Prominence* of a voter

$$Q_j = \log nr_j.$$

24. b_j is a *significant voter* if $Q_j \geq I(B)$.
25. b_j is a *dominant voter* if he is the only significant voter.
26. b_j is a *dictator* if he has all the votes at his disposal without exception.
27. An election is a *passive election* if $I(AB)=0$.
28. An election is called a *dictatorial election* if

$$I(A+B) = \log mn.$$

29. An election is called a *positive election* if there is an eminent candidate.
30. An election is called a *definite election* if there is an outstanding candidate.
31. *Societal preference distribution* $[s_i]$ is the preference chosen by the voting system for the candidates.
32. *Societal injustice* to a candidate

$$J_i = \log(q_i/s_i).$$

33. Societal injustice

$$J = \sum_{i=1}^{m} q_i \log(q_i/s_i).$$

It can be shown that J can never be negative. Note that H is a function of distributions and

$$H[p_1 p_2 .. p_k] = -\sum_{i=1}^{k} p_i \log p_i.$$

H is called entropy in information theory and it is well known that there is no alternative but to have this definition[4] if H is to satisfy certain mild conditions. A possible set of such conditions is

i) H is an analytic function with $H[.5 \quad .5]=1$
ii) $H[p_1 p_2 .. p_k 0] = H[p_1 p_2 .. p_k]$
iii) $H[p_{ij}] = H[r_j] + \sum_{j=1}^{n} r_j H_j[q_{ij}]$.

We mention this as an aside only to indicate how powerful the concept of entropy can be in psephology. It can be shown[4] that H is non-negative and attains a maximum when all the masses are equal and it is zero when one of the masses equals one. The following lemmas can be easily proved making use of these facts and hence the proofs are omitted.

Lemmas

1. $P_i=0$ if and only if a_i gets exactly the average number of votes. P_i is positive or negative depending upon whether a_i collects above or below the average number of votes. $P_i=\log m$, if and only if a_i is a charismatic candidate. The range of P_i is $[-\infty, \log m]$.
2. The hierarchy of the candidates is: charismatic, eminent, outstanding, favourite and popular, i.e. each of these classes implies the classes that follow.
3. In any election there is at least one favourite candidate.
4. A positive election is always a definite election.
5. $I(A)=0$ if and only if all the candidates collect equal votes. $I(A)=\log m$ if and only if there is a charismatic candidate.
6. $Q_j=0$ if and only if b_j has exactly the average number of votes at his disposal. Q_j is positive or negative depending upon whether b_j has above or below the average number of votes. $Q_j=\log n$ if and only if b_j is a dictator. The range of Q_j is $[-\infty, \log n]$.
7. The hierarchy of voters is: dictator, dominant and significant.
8. In any election there is at least one significant voter.
9. $I(B)=0$ if and only if all the voters have equal votes. $I(B)=\log n$ if and only if there is a dictator.
10. $I(A\bar{B})=0$ if and only if each individual voter has given equal votes to all the candidates. $I(A\bar{B})=\log m$ if and only if each individual voter has given all his votes to a single candidate. The range of $I(A\bar{B})$ is $[0, \log m]$.

11. $I(A\bar{B})=0$ if and only if every candidate has received the same number of votes from each voter. $I(A\bar{B})=\log n$ if and only if every candidate has got all his votes from a single voter. The range of $I(A\bar{B})$ is $[0, \log n]$.
12. $I(A+B)=0$ if and only if each candidate has received the same number of votes from each voter. $I(A+B)=\log mn$ if and only if there is charismatic candidate and a dictator. The range of $I(A+B)$ is $[0, \log mn]$.
13. Let $\min\{m,n\}=m$. $H(AB)=\log m$, if and only if, each voter has given all his votes to one candidate and all candidates have collected equal votes.
14. Let $\min\{m,n\}=n$. $H(AB)=\log n$, if and only if, all voters have equal votes and each candidate has collected all votes from a single voter.

The above two lemmas can be proved if we recognize that $H(AB)$ cannot be greater than $\min\{H(A), H(B)\}$.

15. $H(AB)=0$, if and only if, all the voters have exactly the same preference distribution for the candidates. The range of $H(AB)$ is $[0, \log h]$ where $h=\min\{m,n\}$.
16. $J_i=0$, if and only if, $s_i=q_i$. J_i is positive or negative, depending upon whether q_i is greater or less than s_i. The range of J_i is $[-\infty, \infty]$.

POSSIBILITY AND JUSTICE THEOREMS

Axiom of unanimity: $(q_{11}=q_{12}=..=q_{1n}=q)\Rightarrow(s_1=q)$. In words, if each individual voter gives the same preference mass to candidate a_1, so does the voting system.

Axiom of independence: $s_i=f(p_{i1}, p_{i2}, .., p_{in})$, i.e. each s_i is the same function of the corresponding row of $[p_{ij}]$. In other words the voting system does not discriminate between the candidates.

Possibility theorem: In FVS it is possible to satisfy the axioms of unanimity and independence and to have a societal preference distribution. Further, this distribution is unique.

$$\begin{bmatrix} p_{11} & p_{12} & \cdots & p_{1n} \\ \dfrac{p_{11}(1-q)}{q(m-1)} & \dfrac{p_{12}(1-q)}{q(m-1)} & \cdots & \dfrac{p_{1n}(1-q)}{q(m-1)} \\ \vdots & \vdots & \vdots & \vdots \\ \dfrac{p_{11}(1-q)}{q(m-1)} & \dfrac{p_{12}(1-q)}{q(m-1)} & \cdots & \dfrac{p_{1n}(1-q)}{q(m-1)} \end{bmatrix}$$

Figure 3. Modified P matrix.

Proof: From the given matrix $[p_{ij}]$, construct another P matrix as given in figure 3, where

$$q=q_1=\sum_{j=1}^{n} p_{1j}.$$

It is easy to see that for the Q matrix corresponding to this new P matrix, we have

$$q_{11}=q_{12}=\therefore=q_{1n}=q.$$

Hence from the axiom of unanimity we conclude that

$$s_1=q=q_1=\sum_{j=1}^{n} p_{1j}.$$

From the axiom of independence we conclude that

$$s_i=q_i=\sum_{j=1}^{n} p_{ij}.$$

Justice theorem: J attains the minimum value zero, if and only if, $[s_i]=[q_i]$, i.e. the only way to make sure that no injustice is done to the candidates is to choose $[q_i]$ as the societal preference distribution.

Proof: We use the method of Langrange multipliers. Consider

$$U=\sum_{i=1}^{m} q_i \log(q_i/s_i)+\lambda \sum_{i=1}^{m} s_i.$$

$$[\partial U/\partial s_i=-(q_i/s_i)\log e+\lambda=0]\Rightarrow$$
$$[(q_i/s_i)=(\lambda/\log e)]\Rightarrow$$

$$\left[\sum_{i=1}^{m} q_i=(\lambda/\log e)\sum_{i=1}^{m} s_i\right]\Rightarrow$$

$$[(\lambda/\log e)=1]\Rightarrow$$
$$s_i=q_i.$$

We have shown the uniqueness of the societal preference distribution through minimization of societal injustice.

Corollary: The range of societal injustice J is $[0, \infty]$. $J=0$ when $[s_i]=[q_i]$. $J=\infty$ when one $s_i=0$

and the corresponding $q_i \neq 0$, i.e. when the society has unreasonably chosen to ignore a candidate totally.

IMPLEMENTATION OF FVS

In FVS it is useful to consider the last candidate a_m as fictitious and the candidate may be named *anarchy*. All the votes of a voter who protests over the election itself will go to the anarchy candidate. If any voter has utilized some but not all his votes, the unutilized votes will go to anarchy. Any voter who absents himself without protesting against the election will be totally ignored by FVS. If one or more candidates get disqualified after the voting has taken place, FVS will delete their names from the contest and consider the marginal preference distributions of individual voters with respect to the remaining candidates. If anarchy wins the election, it is an indication of the existence of a substantial group of disgruntled citizens who do not want to participate in the democratic process and the breakdown of democracy. It is interesting to note that FVS caters even to this group of people and FVS is generous enough to listen to everybody and repeat the well-known Russel's paradox: What should a true democrat do when the majority says that they do not want democracy.

AN ILLUSTRATIVE EXAMPLE

The matrix shown in figure 4 shows an example in which $N=32$, $m=4$ and $n=8$.

$$[p_{ij}]=\frac{1}{32}\begin{bmatrix} 1 & 2 & 0 & 0 & 0 & 0 & 0 & 1 \\ 2 & 0 & 4 & 0 & 8 & 2 & 0 & 0 \\ 1 & 0 & 0 & 4 & 0 & 1 & 0 & 2 \\ 0 & 0 & 0 & 0 & 0 & 1 & 2 & 1 \end{bmatrix} \qquad [q_i]=\frac{1}{32}\begin{bmatrix} 4 \\ 16 \\ 8 \\ 4 \end{bmatrix}$$

$$[r_j]=\frac{1}{32}\,[4 \quad 2 \quad 4 \quad 4 \quad 8 \quad 4 \quad 2 \quad 4]$$

Figure 4. An example.

Each of the voters b_6 and b_8 used three of their votes even though each of them had four votes at their disposal, hence their unused votes have gone to anarchy a_4. Voter b_7 had protested against the election and hence his two votes have been given to anarchy. In this election a_2 gets the highest number of votes, namely 16, and hence he gets elected.

Voter preference

$I_1(A)=1/2,\ I_2(A)=2,\ I_3(A)=2,\ I_4(A)=2,$
$I_5(A)=2,\ I_6(A)=1/2,\ I_7(A)=2,\ I_8(A)=1/2.$

Conditional preference

$I(A\bar{B})=23/16.$

Panel heterogeneity

$I(A)=1/4.$

Candidate popularity

$P_1=-1,\ P_2=1,\ P_3=0,\ P_4=-1.$

a_2 and a_3 are popular candidates and a_2 is an outstanding candidate.

Clan affinity

$I_1(B)=3/2,\ I_2(B)=5/4,\ I_3(B)=5/4,\ I_4(B)=3/4.$

Conditional affinity

$I(AB)=21/16.$

Electorate heterogeneity

$I(B)=1/8.$

Voter prominence

$Q_1=0,\ Q_2=-1,\ Q_3=0,\ Q_4=0,\ Q_5=1,\ Q_6=0,$ $Q_7=-1,\ Q_8=0$; b_5 is a dominant voter.

Societal heterogeneity

$I(A+B)=25/16.$

Election campaign

$H(AB)=19/16.$

CONCLUSION AND RECOMMENDATION

Consider the Presidential election system in India where the single transferable vote (STV) is used at present. As a hypothetical case assume that the total value of the votes of all the Members of Parliament is 1001. Imagine an election in which 501 votes are in favour of the preference order ABCDE and the rest 500 votes in favour of the preference order BCDEA. If this situation arises, STV will choose *A* as the President, which is obviously a wrong choice especially because half the electorate hate *A*. Hence it is recommended that for the Presidential election the present system of STV be discarded in favour of the FVS proposed here. From the possibility and justice theorems given earlier it should be clear that anomalous situations cannot occur with FVS.

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ANNOUNCEMENTS

National Symposium on Bioactive Compounds from Marine Organisms

Place: Parangipettai
Date: December 1989
Contact: Prof. A. Subramanian
Centre of Advanced Study in Marine Biology
Parangipettai 608 502

National Symposium on Conservation and Management of Living Resources

Place: Bangalore
Date: 10–12 January 1990
Contact: Dr Shakunthala Sridhara
Organizing Secretary
National Symposium on Conservation and Management of Living Resources
University of Agricultural Sciences
GKVK, Bangalore 560 065

National Symposium on Environmental Influences on Seed and Germination Mechanism—Recent Advances in Research and Technology

Place: Jodhpur
Date: 27–29 January 1990
Contact: Prof. David N. Sen
Department of Botany
University of Jodhpur
Jodhpur 342 001

First International Conference on Vibration Problems of Mathematical Elasticity and Physics

Place: Jalpaiguri, India
Date: 20–23 October 1990
Contact: Dr M. M. Banerjee/Dr P. Biswas
Vibration Conference Secretariat
Department of Mathematics
A.C. College
Jalpaiguri 735 101
India
Abstracts by 1 February 1990

XXI National Seminar on Crystallography

Place: Bombay
Date: 27–29 December 1989
Contact: The Secretary
XXI National Seminar on Crystallography
Neutron Physics Division
Bhabha Atomic Research Centre
Bombay 400 085

National Seminar on Aquatic Pollution

Place: Trivandrum
Date: 18–20 December 1989
Contact: Dr P. K. Abdul Azis
Organizing Secretary
National Seminar on Aquatic Pollution
Department of Aquatic Biology and Fisheries
Trivandrum 695 007

MECHANISM OF Ru(III) CATALYSIS IN ACID BROMATE OXIDATION OF SOME GLYCOLS: A KINETIC STUDY

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ABSTRACT

Ru(III)-catalysed oxidation of methyldiethyleneglycol and ethyldiethyleneglycol by acidic solution of potassium bromate shows zero-order dependence on bromate and H^+ ions and first-order dependence on Ru(III) and each glycol. The corresponding aldehyde is the reaction product. The proposed mechanism involves slow and rate-controlling disproportionation of a transient complex formed between reactive species of Ru(III) and glycol. Various activation parameters have been computed.

INTRODUCTION

POTASSIUM bromate, being a powerful oxidant with a redox potential of 1.44 V in acidic media, has been widely used in the oxidation of alcohols[1], cyclanols[2,3], phenols[4], α-hydroxy acids[5], aldehydes[6-8], tartaric acid[9] and some labile substrates[10]. The literature on the role of Ru(III) chloride as catalyst in acid bromate oxidations is scanty. Only oxidative kinetics of a few ketones with bromate as oxidant and Ru(III) as catalyst have been reported[11] and mechanistic interpretations of the results are obscure. This prompted us to undertake the present investigation of the kinetics and mechanism of Ru(III)-catalysed oxidation of methyldiethyleneglycol (MDG) and ethyldiethyleneglycol (EDG) by acidic solution of potassium bromate in the presence of mercuric acetate as bromide ion scavenger.

EXPERIMENTAL

Materials and procedures

Potassium bromate (BDH, AR) was used as such. E. Merck samples of sodium perchlorate, perchloric acid (60%) and mercuric acetate were used. The other reagents used were of the highest purity available. Solutions of the glycols (BDH, LR) were prepared by weighing the samples. Ru(III) chloride (Johnson Matthey) solution was prepared by dissolving the sample in hydrochloric acid of known strength. All the solutions were prepared in triple-distilled water.

The reaction stills were blackened from the outside to avoid any photochemical reactions. All reactants except glycol were allowed to mix and the reaction was initiated by the rapid addition of an appropriate amount of glycol to the already equilibrated mixture of reactants. The progress of the reaction was monitored by estimating the amount of remaining bromate iodometrically. All the observations were taken at constant temperature (±0.1°C).

RESULTS AND DISCUSSION

Stoichiometric data indicated consumption of one mole of bromate in the oxidation of each mole of glycol. The corresponding aldehydes were confirmed as end products by TLC and also by preparation of the dinitrophenylhydrazine (DNP) derivative[12]. Mercury(II) acetate[13] was used as bromide ion scavenger and it did not interfere with the reaction in any way. Zero-order dependence on bromate and first-order on each glycol were obvious from the data (table 1). Zero-order rate constants ($-dc/dt$ or k_0) were calculated from slopes of plots of unconsumed bromate versus time for different initial concentrations of bromate. First-order kinetics in Ru(III) and zero-order dependence on H^+ are also obvious from the results (table 2). Successive addition of mercuric acetate, chloride ions and sodium perchlorate (ionic strength variation) had insignificant effect. Successive addition of acetic acid increased the reaction rate, showing a negative dielectric effect on the rate (table 2). The values of activation parameters, computed from the rate at 30, 35, 40 and 45°C, are given in table 3.

Table 1 *Effect of concentration of reactants on reaction rate in acid bromate oxidation of glycols*
[Ru(III)] = 1.92×10^{-5} M (unless otherwise stated), [$HClO_4$] = 1.00×10^{-3} M, [$Hg(OAc)_2$] = 3.34×10^{-3} M at 35°C.

[Bromate] (10^{-3} M)	[Substrate] (10^{-2} M)	$\left(-\frac{dc}{dt}\right) \times 10^7$ (mol l^{-1} s^{-1}) MDG	$\left(-\frac{dc}{dt}\right) \times 10^7$ (mol l^{-1} s^{-1}) EDG
0.67	3.34	3.16	3.16[a]
0.83	3.34	3.00	2.86[a]
1.00	3.34	3.36	2.66[a]
1.33	3.34	3.33	2.76[a]
2.22	3.34	2.83	2.85[a]
3.33	3.34	3.50	3.16[a]
1.00	1.00	0.53[b]	1.16[c]
1.00	1.66	0.88[b]	—
1.00	2.22	1.03[b]	2.58[c]
1.00	3.34	2.00[b]	4.00[c]
1.00	5.00	2.75[b]	5.83[c]
1.00	10.00	5.33[b]	12.08[c]
1.00	20.00	—	23.34[c]

[a]At [EDG] = 5.00×10^{-2} M; [b]at 30°C; [c]at [Ru(III)] = 3.84×10^{-5} M.

Table 2 *Effect of variation of concentration of Ru(III) and perchloric acid on reaction rate at 35°C*
[BROMATE] = 1.00×10^{-3} M, [MDG] = 5.00×10^{-2} M, [EDG] = 5.00×10^{-2} M (unless otherwise stated), and [$Hg(OAc)_2$] = 1.25×10^{-3} M.

[Ru(III)] 10^6 M	[$HClO_4$] 10^3 M	$\left(-\frac{dc}{dt}\right) \times 10^7$ (mol l^{-1} s^{-1}) MDG		EDG	
3.84	1.00	—		0.58	
5.76	1.00	—		0.81	
7.68	1.00	1.64	4.30[a]	0.15	6.00[e]
13.44	1.00	2.85	6.29[b]	—	7.83[f]
15.36	1.00	3.27	8.80[c]	2.32	13.83[g]
19.20	1.00	4.10	21.66[d]	2.92	23.33[h]
28.80	1.00	6.13		—	
38.40	1.00	8.00		5.83	
19.20	0.40	4.86		2.88	
19.20	0.50	4.72		2.90	
19.20	0.67	4.10		2.96	
19.20	1.00	4.66		2.89	
19.20	1.25	4.70		2.86	
19.20	2.00	4.86		2.92	
19.20	4.00	4.83		2.90	

[Acetic acid] = 5 (a, e), 10 (b, f), 20 (c, g) and 30% (d, h) at [Ru(III)] = 19.20×10^{-6} M and (e, f, g, h) [EDG] = 2.22×10^{-2} M.

The number of possible chloro species of Ru(III) present in solution is given by $[Ru(III)\,(6-n)(H_2O)\,Cl_n]^{3-n}$, where $n = 1$–6. All these species are pH-dependent. The species present in the pH range[14,15] studied here is, however, $[RuCl_2(H_2O)_4]^+$. The absence of any effect on the reaction rate upon addition of chloride ions rules out the possibility of either dissociation or association of Cl^- ions. Hence $[RuCl_2(H_2O)_4]^+$ is proposed as the reactive species of Ru(III) chloride in the employed pH range.

Table 3 *Activation parameters for bromate oxidation of glycols*

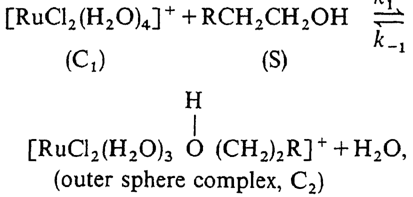
Parameter	MDG	EDG
10 kr (30°C) (mol^{-1} l s^{-1})	2.86	2.17
10 kr (35°C) (mol^{-1} l s^{-1})	4.29	3.04
10 kr (40°C) (mol^{-1} l s^{-1})	6.09	4.41
10 kr (45°C) (mol^{-1} l s^{-1})	9.00	7.50
ΔE^* (kJ mol^{-1})	63.84	69.60
log A	10.45	11.27
ΔS^* (JK^{-1} mol^{-1})	−48.84[a]	−33.13[a]
ΔF^* (kJ mol^{-1})	78.88[a]	79.80[a]

[a]At 35°C.

In acidic media bromate[9] ions form $HBrO_3$ according to equation (1):

$$BrO_3^- + H^+ \rightleftharpoons HBrO_3. \qquad (1)$$

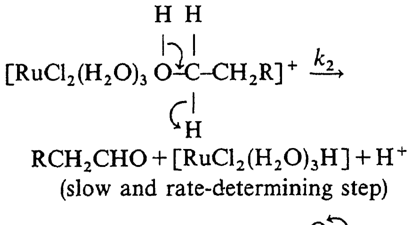
The zero-order dependence on both bromate and H^+ indicates that $HBrO_3$ formed in (1) acts as an oxidant and is involved in the fast step. On the basis of these statements and kinetic results the following reaction routes are suggested:



$$\underset{(C_1)}{[RuCl_2(H_2O)_4]^+} + \underset{(S)}{RCH_2CH_2OH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [RuCl_2(H_2O)_3\,\overset{\overset{H}{|}}{O}\,(CH_2)_2R]^+ + H_2O, \qquad (I)$$

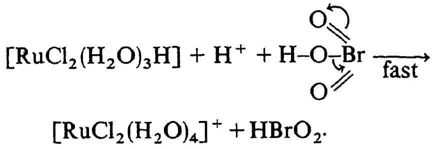
(outer sphere complex, C_2)

where R stands for $-OCH_2CH_2OR'$ and R′ for either $-CH_3$ or $-C_2H_5$ group in MDG or EDG.



$$[RuCl_2(H_2O)_3\,\overset{\overset{H}{|}}{O}-\overset{\overset{H}{|}}{\underset{\underset{H}{|}}{C}}-CH_2R]^+ \xrightarrow{k_2} RCH_2CHO + [RuCl_2(H_2O)_3H] + H^+ \qquad (II)$$

(slow and rate-determining step)



$$[RuCl_2(H_2O)_3H] + H^+ + H-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{\|}}{Br}} \xrightarrow[\text{fast}]{} [RuCl_2(H_2O)_4]^+ + HBrO_2. \qquad (III)$$

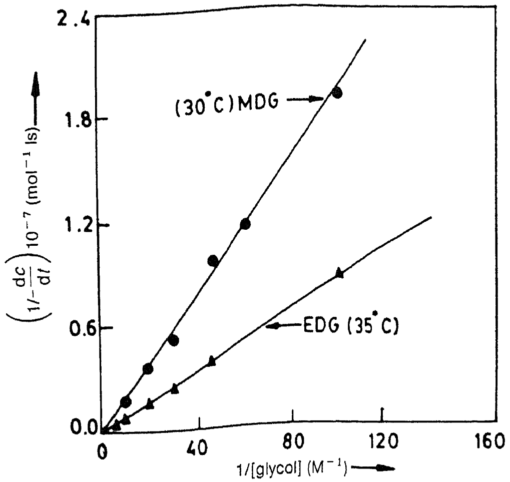


Figure 1. (1/rate) vs 1/[glycol] plots for MDG and EDG. [Bromate] = 1.00×10^{-3} M, [$HClO_4$] = 1.00×10^{-3} M, [Ru(III)] = 1.92×10^{-5} M (MDG) 3.84×10^{-5} M (EDG), [$Hg(OAc)_2$] = 3.34×10^{-3} M.

Considering steps (I) and (II) and applying steady-state treatment to [C_2], the rate of the reaction in terms of consumption of bromate may be expressed as equation (2) below, with limiting conditions $k_{-1} \gg k_2$ and $1 \gg K_1([S]/[H_2O])$, where K_1 is the complex formation constant and is equal to k_1/k_{-1}.

$$-\frac{d\,[BrO_3^-]}{dt} = k_0\,(\text{obs.}) = k_2K_1\,[S]\,[Ru(III)]_T \quad (2)$$

The rate law (2) explains all the kinetic observations. The hydride ion transfer mechanism also finds support from Bailer *et al.*[16], and Ru(III)–alcohol complex formation is well established[17,18]. Plots of $1/(-dc/dt)$ vs 1/[glycol] are linear and pass through the origin, indicating that the complex has a low stability constant[19] (figure 1).

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SHORT COMMUNICATIONS

SUPERCONDUCTIVE BEHAVIOUR OF $Ba_2Y_{0.5}La_{0.5}Cu_3O_{7-\delta}$

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SINCE Bednorz and Muller[1] reported the possible existence of percolative superconductivity in the La–Ba–Cu–O system in the 30 K range, some new systems have been reported as high-temperature superconducting materials. Basically the currently available high T_c superconductor can be classified under two categories, viz. K_2NiF_4 type crystal structure ($T_c = 20 \sim 50$ K) and $Ba_2YCu_3O_{7-\delta}$ type crystal structure ($T_c = 70 \sim 100$ K). At present the mechanism of high T_c superconductivity is a challenging problem and much work has to be done in both theoretical and experimental fields. A study of the compositional and structural dependence of superconductivity is important to substantiate the mechanism of high T_c superconductivity in Cu-containing oxides and to prepare new high T_c materials. In an attempt to understand the problem we have studied the system $Ba_2Y_{0.5}La_{0.5}Cu_3O_{7-\delta}$ carefully by comparing the results of X-ray diffraction, electrical resistivity and AC susceptibility. The pressure shift of T_c for the compound is also shown.

Samples of $YBa_2Cu_3O_{7-\delta}$ and $LaBa_2Cu_3O_{7-\delta}$ were prepared from the appropriate mixtures of $BaCO_3Y_2O_3$, La_2O_3 and CuO. The starting materials were thoroughly mixed, ground and then heated to 900°C for 12 h in air to prepare the master materials. These materials were reground to get fine powder. The mixture of the two master materials in the requisite proportion was pressed into a disc and sintered at 900°C for 15 h in air. Then the sample was slowly furnace-cooled to 200°C over a period of 6 h. All samples were prepared under the same conditions.

The superconductor oxide with a metal ratio Y:Ba:La:Cu of 0.5:2.0:0.5:3.0 showed an X-ray diffraction pattern similar to that of the distorted perovskite structure. The X-ray diffraction pattern was used to check for the presence of secondary phases. The lattice parameters of the title compound are as follows: $a = 3.855$ Å, $b = 3.914$ Å and $c = 11.811$ Å. These values compare well with the values reported in the literature [$a = 3.848$ Å, $b = 3.899$ Å $c = 11.77$ Å (ref. 2); $(b-a)/a = 14 \times 10^{-3}$, $c = 11.72$ Å, $T_c = 94 \sim 98$ K (ref. 3)]. When $x \geqslant 0.9$ (in $Ba_2Y_{1-x}La_xCu_3O_{7-\delta}$), the crystal structure changes from orthorhombic to tetragonal; that is, samples with x in the range 0.90–0.99 are semiconductors. Resistivity of the superconductor was determined in samples cut from the disc. Pressure shift of T_c was determined by measuring the electrical resistance as a function of temperature at various pressures. Copper wires were attached to the specimen by silver paste for making electrical resistance measurements using the four-probe technique. Kerosene was used as pressure transmitting medium, which ensured hydrostatic pressure over the measuring temperature range. The specimen was immersed in kerosene with a teflon capsule. Pressure was applied by using a piston–cylinder-type press.

The temperature dependence of electrical resistance was measured under pressures of 0, 1, 2, 3 and 4 GPa. The resistance vs temperature graphs at 0, 1, 2, 3 and 4 GPa are shown in figure 1.

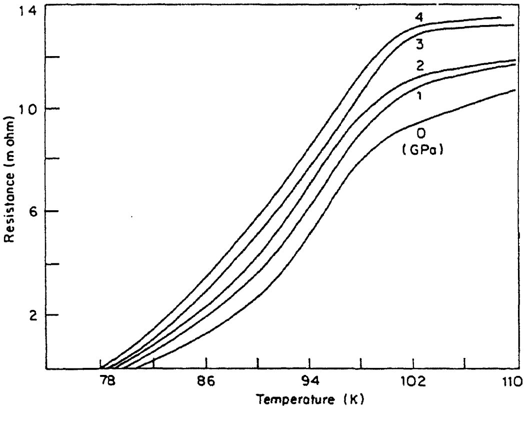

Figure 1. Electrical resistance of $Ba_2Y_{0.5}La_{0.5}Cu_3O_{7-\delta}$ vs temperature at 0, 1, 2, 3 and 4 GPa.

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*For correspondence

The following observations are made from figure 1:

(i) All resistance vs temperature curves have a slight shoulder in the transition temperature range
(ii) The pressure shift of T_c (onset) T_{co} and T_c (final) T_{cf} is very small
(iii) The superconducting transition of the title compound terminates at 77 K at atmospheric pressure
(iv) (dR/dP) changes with increasing pressure.

The variation of transition temperature with pressure is shown in figure 2. T_{co} and T_{cf} were determined and plotted to observe the pressure dependence.

The pressure dependence of T_{co} and T_{cf} is not so large when compared with that in the La–Ba–Cu–O system. However, T_{cf} shows a maximum at 2, 3 and 4 GPa (figure 2).

The AC susceptibility down to 5 K was determined using the closed cycle refrigerator. The variation of χ_{ac} with temperature is shown in figure 3. The bulk sample exhibits a large diamagnetic signal at temperatures below 98 K. When the sample was powdered, the diamagnetic signal was reduced to 1/4 of the signal for the bulk sample at 5 K. This implies that the sample possesses superconducting phase at $T = 98$ K only on the surface and/or given boundaries[4]. It is also interesting to note that even for a solid pellet the diamagnetic susceptibility reaches a limiting value only at 30 K though the resistivity drops to zero at 77 K.

The interesting feature in figure 1 is the resistance behaviour in the normal conducting state. The transition temperature changes with increasing pressure. The positive sign of (dR/dT) at low pressures becomes negative at a certain pressure between 1 GPa and 2 GPa. T_{co} shows a faint maximum around 1 GPa as a whole. This maximum corresponds to the pressure at which the sign (dR/dT) in the normal state changes from positive to negative as mentioned earlier. Similar behaviour was also noticed by Akahama *et al.*[5]

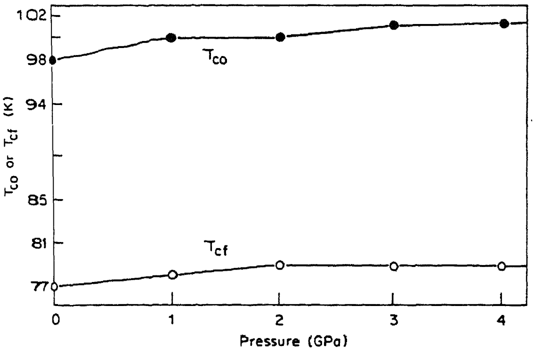

Figure 2. Pressure dependence of T_{co} and T_{cf} for $Ba_2Y_{0.5}La_{0.5}Cu_3O_{7-\delta}$.

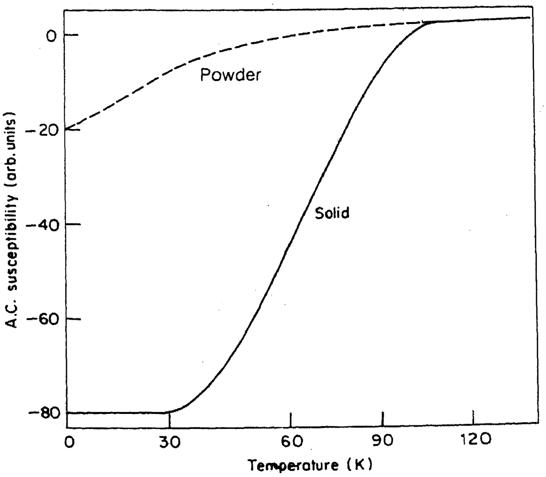

Figure 3. Variation of χ_{ac} with temperature for $Ba_2Y_{0.5}La_{0.5}Cu_3O_{7-\delta}$.

Preparation of samples with different compositions and high pressure studies are in progress.

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WHY HAS URANUS TOPPLED OVER?

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URANUS is unique among the planets in the solar system because its spin axis lies in its orbital plane. Whereas the spin axes of the neighbouring Saturn and Neptune show a tilt of 27° and 29° from the normal to their orbital planes (similar to the case of

Earth and Mars), the tilt is 98° in the case of Uranus. We argue here that the peculiar rotation of Uranus is due to a tidal encounter of proto-Uranus with a massive passing planetary body in the early solar system, that may still be deleted as the tenth planet.

In analogy with the specific models for Jupiter and Saturn, one presumes that, irrespective of the actual mechanism of their formation, the outer Jovians Uranus and Neptune have also passed through three major phases from their origin to the present[1].

The first phase was characterized by a slow contraction of the planetary mass, on a Kelvin–Helmholtz time-scale of a few million years. In the second phase, the proto-planet underwent rapid collapse on a free-fall time-scale of a few years. During this phase, the shrinking proto-planet shed an equatorial disc, from which the satellite system formed. The third phase again involved slow contraction and is still continuing.

The present-day parameters of Uranus are as follows: mass 8.7×10^{28} g, equatorial radius 2.5×10^9 cm, oblateness 0.024, rotation period 17.24 h, angular momentum $\sim 2 \times 10^{43}$ g cm^2 s^{-1}. Our starting point however is an extended proto-Uranus that is yet to go into free fall. It is slowly rotating about an axis that, like in Saturn and Neptune, has an unexplained yet acceptable tilt of 28°.

At this stage the proto-planet of mass M and semi-major axes $a_1 = a_2 > a_3$ undergoes a tidal encounter with a planetary body of mass M' passing by with a velocity $\mathbf{v}$ and at a pericentric distance $\mathbf{p}$. The perturber transfers to proto-Uranus an angular momentum whose components are[2]

$$J_i = \frac{2GM'}{p^2 v}(I_{kk} - I_{jj})\alpha_{jk}. \tag{1}$$

Here the indices i, j, k cyclically take the values 1, 2, 3. I_{ij} is the moment of inertia tensor of the proto-planet. $\alpha_{jk} = 2\hat{p}_j\hat{p}_k + \hat{v}_j\hat{v}_k - \delta_{jk}$, with $\hat{\mathbf{p}}$, $\hat{\mathbf{v}}$ being unit vectors along $\mathbf{p}$, $\mathbf{v}$ respectively: $\mathbf{p} \cdot \mathbf{v} = 0$.

Equation (1) has been derived using impulse approximation[3] and generalizes Peebles' well-known result[4] derived in the context of proto-galaxies. Note from equation (1) that, if the proto-planet is axisymmetric, the transferred angular momentum would be about an axis in the equatorial plane:

$$J = \frac{1}{5} e^2 GM \left(\frac{a_1}{p}\right)^2 \frac{M'}{v}, \tag{2}$$

where we have ignored the angular dependences. Here e is the eccentricity of the proto-planet: $a_3^2 = a_1^2(1 - e^2)$. If this tidally introduced angular momentum is about three times the initial angular momentum, which is about the perpendicular axis, then the net angular momentum would make an angle of 70° with the original direction so that, now, rotation would be about an axis tilted at 98° to the normal to the orbital plane. It is this axis that would be the new axis of symmetry, and satellite disc would form in the new equatorial plane.

There are no clues to the parameters of the tidal encounter. The proto-planetary mass however would not have been much different from the present-day Uranian mass. (It would have been slightly higher, see below.) If proto-Uranus had an eccentricity of 0.7 (corresponding to an oblateness of 0.3), and if the encounter was a grazing one: $p = 2a_1$, we can write from equation (2),

$$J = 4 \times 10^{42} \frac{M'/M_E}{v/\mathrm{kms}^{-1}} \mathrm{g\,cm}^2\,\mathrm{s}^{-1}, \tag{3}$$

where M_E is the mass of the earth.

If this angular momentum is to correspond to the present-day angular momentum of Uranus, the dimensional parameter M'/v should have a numerical value of 5. Thus the perturbing body could have been a $5M_E$ planetary body moving with a velocity of 1 km s^{-1}; or even a $100M_E$ body with a velocity of about 20 km s^{-1}. The tidal encounter would add to the internal energy of proto-Uranus[5]

$$\frac{\delta \mathrm{U}}{|\mathrm{U}|} = \frac{GM'^2}{p v^2 M}\left(\frac{a_1}{p}\right)^3.$$

Assuming the pericentric distance p to be twice the proto-planetary equatorial radius, which in turn is set equal to ten times the present Uranian radius, we see that $\frac{\delta \mathrm{U}}{|\mathrm{U}|} \sim 1$, so that proto-Uranus would not be disrupted. The outer layers of proto-Uranus may however be torn off as a result of this close, though brief, encounter.

The outer Jovians are indeed enigmatic. Mostly rock and ices, both Uranus and Neptune are short of hydrogen and helium gases. Uranus has peculiar rotational properties, even though its satellite system is normal. On the other hand, while Neptune itself is normal, it has but two pre-Voyager2 moons, both irregular. Additionally, the Pluto–Charon system may be displaced Neptunian moons.

Already the tilted spin axis of Uranus has been attributed to the impact of an $\sim 2M_E$ planetary body on proto-Uranus[6]. Numerical experiments[7] have shown that the properties of the present Neptunian moons and the Pluto–Charon system can be understood in terms of the tidal encounter of Neptune with a 2–5 M_E planetary body.

The idea of a perturbing body (with a dimensional mass/velocity of 5) in the outskirts of the solar system has many attractions. As we have seen, it can explain the tilt of the Uranian spin axis. This perturbing body would take 10–100 years (depending upon its velocity) to move to the Neptunian orbit. If during this time Neptune has already formed its equatorial disc, then the tidal effect of this outgoing can explain why the two Neptunian satellites became irregular and Pluto–Charon heliocentric. And if the body had a mass of $100M_E$ it can account for the missing gas that should otherwise have enriched the outer Jovians.

If the perturber had a very small velocity (and a low mass), it would end up as a satellite of Uranus. This is ruled out because of the extreme regularity of the Uranian satellite system, which precludes a captured satellite. If the perturber had a velocity of about 7 km s^{-1}, it would be bound in the Sun's gravitational potential, and should be detectable as a yet undiscovered planet at a large heliocentric distance.

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BEDDING PLANE FAULT IN THE KALADGI ROCKS, BASIDONI, BELGAUM DISTRICT, KARNATAKA STATE

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DEVELOPMENT of a bedding-plane fault in the quartzarenitic rocks of the Kaladgi Group at Basidoni (16°51′25″N, 75°13′30″E), situated 18 km north-east of Saundatti, is evidenced by the development of a bluish-green pseudotachylyte, covering an area of 4500 m^2. The layer of pseudotachylyte is sandwiched between low-dipping quartzarenites. Striations and grooves trending N 70°E–S 70°W and N 30°W–S 30°E, and shears trending N 35°W–S 35°E (most frequent), N 70°E–S 70°W (intermediate frequency) and N 70°W–S 70°E (least frequent) are associated with the bedding-plane fault.

Several workers[1–5] have studied different parts of the Kaladgi basin, but there is no mention of such a fault by them. Angular unconformity is clearly noticed between the Kaladgi and the underlying Archaean gneisses. Two kilometres north-west of Basidoni, exposures of 80 m-thick pink quartzarenites dipping 4–5° due S 20°E are characterized by the presence of a thin (1 cm) veneer of glassy, bluish-green pseudotachylyte (figure 1). The bedding-plane fault dips gently (2–3°) due south-east. Pseudotachylyte layers are also noticed in the beds of quartzarenites occurring below the one shown in figure 1. In the vicinity of psuedotachylyte the rocks are found to be actively sheared in several directions.

The development of pseudotachylyte in the quartzarenites and the association of striations, grooves and shear planes in the vicinity of psuedotachylyte support the existence of a bedding-plane fault. The striations indicate displacement on the fault plane in two directions, viz. N 70°E–S 70°W and N 30°W–S 30°E. However, considering the higher frequency of striations trending N 70°E–S 70°W, it is suggested that greater/frequent movement might have taken place in this direction. Considering the fact that striations trending N 30°W–S 30°E are obscure, it may be surmised that the movement in that direction occurred at an earlier period and consequently the striations have become obscure.

The development of several parallel layers of pseudotachylyte one below the other probably indicates the presence of several 'parallel bedding-plane faults'.

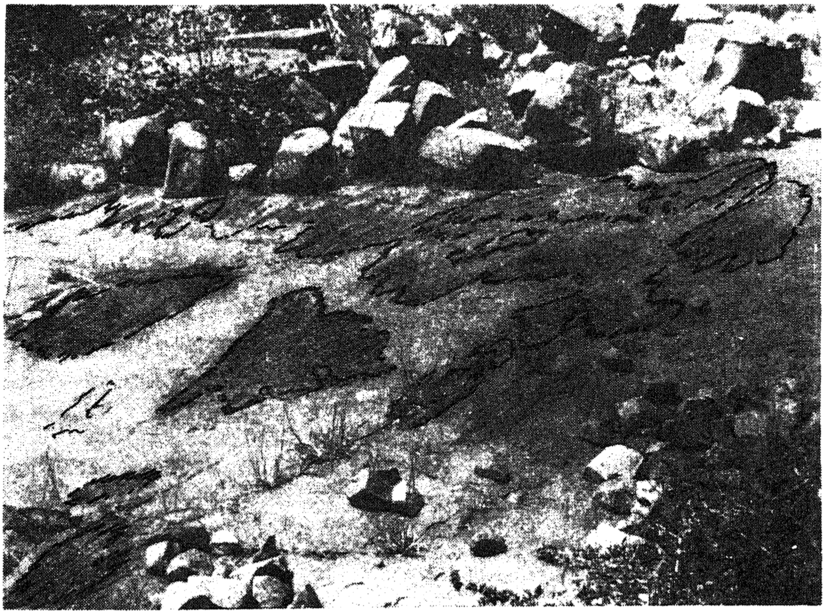

Figure 1. Field photo showing the development of unusually large-sized bedding fault. Note the development of bluish-green pseudotachylyte, the remnants of which are seen in the form of patches (outlined). Striations in two different directions are also developed, though they are obscured. View looking north-west. Location: 2 km west of Basidoni village.

One of the authors (GSP) thanks the Karnatak University, Dharwad, for award of URS.

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REACTION OF DIBENZOYLMETHANE WITH FORMALDEHYDE: A REVISED STRUCTURE FOR THE PRODUCT

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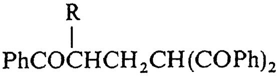
RECENTLY, Joglekar and Samant[1] reported that 1,3-diphenyl-1,3-propanedione and formaldehyde in ethanol in presence of different amines gave 2-benzoyl-1,5-diphenyl-1,5-pentanedione (**1**) through base-catalysed cleavage. Spectroscopic data were interpreted and a suitable mechanism for the formation of **1** has also been proposed. However, a critical examination of the PMR spectroscopic data of **1** points out that (i) the methylene (4-CH_2) adjacent to carbonyl appears at considerably downfield (5.70 ppm), which is unusual for this type of protons[2], and (ii) the absence of a signal corresponding to a methine (2-CH) connected to two benzoyl groups, although such protons were located earlier[3].

A literature survey reveals that the PMR data reported for **1** by Joglekar and Samant agree well with those recorded for 2,4-dibenzoyl-1,5-diphenyl-1,5-pentanedione (**2**)[3,4].



$$\overset{\displaystyle R}{\overset{|}{\text{PhCOCH}}}\text{CH}_2\text{CH(COPh)}_2$$

1 R = H

2 R = COPh

The mass spectrum of **1** was reported[1] to have a peak at m/z 356, attributed to the molecular ion. It is known that compounds of type **2** fragment extensively[2] at 70 eV and the molecular ions may not be observed.

In view of the above, the reaction was repeated under typical conditions reported[1], using piperidine as the base. A product with the same m.p. and other data reported for **1** was obtained. However, the ^{13}C NMR spectrum of the product showed two upfield signals at δ29.1(*t*) and 54.2(*d*) only, suggesting that the structure of the product is **2** and not **1**. In fact, the mass spectrum recorded in CI mode using methane as ionizing gas gave the ion at *m/z* 461, corresponding to $(M+H)^+$ of **2**.

From the foregoing, it is necessary to revise the structure of the product in the title reaction to **2**. It is interesting to note that the reaction of dibenzoylmethane and formaldehyde in ether in presence of diethylamine was reported to give 1,1-dibenzoylethylene[5], but revised latter to **2**[3,4]. Formation of **2** was also favoured by Lieberman and Wagner[6] in both acid- and base-catalysed reactions, but without any spectroscopic evidence.

Experimental

A mixture of 1,3-diphenyl-1,3-propanedione (1.12 g, 0.005 mol), formaldehyde (35% aq. solution, 1 ml, 0.01 mol), piperidine (0.5 ml) and ethanol (20 ml) was heated on a steam bath for 20 min and the reaction mixture was then kept overnight at room temperature. The precipitated solid was recrystallized from ethanol.

Yield: 0.69 g (60%), m.p. 178–180°C. IR (KBr): 1688 and 1668 cm^{-1}. PMR ($CDCl_3$, 90 MHz): δ 2.78 (*t*, $J = 7.5$ Hz, 2 H), 5.75 (*t*, $J = 7.5$ Hz, 2 H), 7.40–7.60 (*m*, 12 H), 8.05–8.20 (*m*, 4 H). CMR ($CDCl_3$, 22.5 MHz): δ 196.7 (*s*), 135.7 (*s*), 134.0 (*d*), 129.1 (*d*), 128.9 (*d*), 54.2 (*d*), 29.1 (*t*). Mass (CI/CH_4): *m/z* (%)—461 (22.2), 369 (22.2), 329 (33.0), 322 (28.7), 253 (29.2), 237 (100.0).

The author thanks Prof. G. S. R. Subba Rao, Indian Institute of Science, Bangalore, for NMR data and Regional Sophisticated Instrumentation Centre, Central Drug Research Institute, Lucknow, for CI mass spectral data.

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CONDENSATION PRODUCTS OF β-PHENYLPROPIONIC ACID WITH COMMERCIAL PPA

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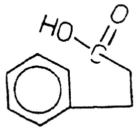
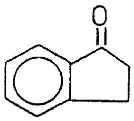
β-PHENYLPROPIONIC acid (I) is cyclized to indan-1-one(II) when the former is heated with commercial polyphosphoric acid at 70°C for 1.5 h. Indan-1-one is one of the starting materials for the synthesis of coumarins[1] and isocoumarins[2,3], two medicinally important class of compounds. During the preparation of indan-1-one by the literature method[1] it was found that when the temperature is raised to 140°C keeping the reaction mixture well stirred for 3.5 h, a product characterized as anhydrobis-indan-1-one(III), is obtained. However, carrying out the



I



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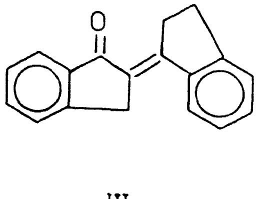
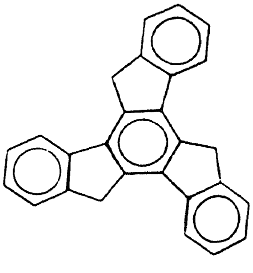


III



IV

reaction at 165°C for 4.5 h, a different product characterized as anhydrotris-indene (IV), was obtained. Both III and IV are formed due to intermolecular cyclodehydration of two and three molecules of indan-1-one (II) respectively.

Anhydrobis-indan-1-one (III): β-Phenylpropionic acid (6 g) and PPA (90 g) were kept at 140°C for 3.5 h with constant stirring. The whole mass was then poured into ice-cold water. The organic portion was extracted with ether (3 × 50 ml), washed with 5% aqueous $NaHCO_3$ solution, water and then dried with Na_2SO_4. Removal of the solvent afforded a solid mass which upon recrystallization with ethyl acetate gave shining yellow crystals (1.97 g, 40%), m.p. 141–42°C; M^+ 245; Found C, 87.78%; H, 5.68%; Calcd. for $C_{18}H_{14}O$ (required: C, 87.8%; H, 5.7%).

ν_{max}^{KBr} 1724, 1675, 1600, 1580, 1500, 1480 cm^{-1}

λ_{max}^{MeOH} 395, 240 nm

δ ppm ($CDCl_3$) 3.16 (t, $Ar\text{–}CH_2$, $-2H$), 3.53 (t, $Ar\text{–}CH_2\text{–}CH_2C=\overset{|}{C}\text{–}$, $-2H$) 3.99 (s, $Ar\text{–}CH_2\text{–}C=C$, $-2H$), 7.41–7.81 (complex, 8H-aromatic).

Anhydrotris-indene (IV): β-Phenylpropionic acid (4.5 g) and PPA (100 g) were kept at 165°C for 4.5 h with constant stirring followed by similar treatments as for (III) afforded deep yellow crystals, which did not melt up to 295°C, M^+ 341; C, 94.72%; H, 5.26% Calcd. for $C_{27}H_{18}$ (required C, 94.73%; H, 5.27%).

ν_{max}^{KBr} 3050, 1600, 1450, 750, 700 cm^{-1}

λ_{max}^{MeOH} 440 nm

δ ppm ($CDCl_3$) 3.8 (s, $Ar\text{–}CH_2\text{–}$, 6H); 7.5–7.9 (complex, 12H aromatic).

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15 December 1988; Revised 17 March 1989

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A SINGLE-STEP SYNTHESIS OF 5- AND 7-SUBSTITUTED 2-(*p*-BROMOBENZOYL)-3-METHYL-4*H*-1, 4-BENZOTHIAZINES

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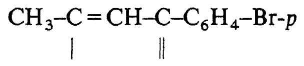
SYNTHESIS of 5- and 7-substituted 2-(*p*-bromobenzoyl)-3-methyl-4*H*-1, 4-benzothiazines by the condensation and oxidative cyclization of 3- and 5-substituted 2-aminobenzenethiols with *p*-bromobenzoylacetone in dimethyl sulphoxide is reported.

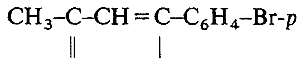
4*H*-1, 4-Benzothiazines resemble phenothiazines in having a fold along nitrogen–sulphur fold, which is the structural feature responsible for biological activity[1]. They constitute an interesting and important class of bioactive molecules. Some derivatives reported have been found to possess anti-inflammatory[2], anthelminthic[3], anti-hypertensive[4], anti-histaminic[5], tranquillizer[6], diuretic[7], lipid regulating[8], spasmolytic[9], anti-bacterial[10], CNS depressant[11] and antiulcer[12] activity.

In continuation of our programme to synthesize novel bio-active molecules[1], the title compounds have been synthesized by one-pot reaction. It involves the condensation of 2-amino-3 and 5-substituted benzenethiols and *p*-bromobenzoylacetone in DMSO which causes oxidative cyclization. The reaction is believed to proceed via the formation of an enaminoketone. 2-Aminobenzenethiols (I) are readily oxidized to disulphides[1,13] (Ia) under the experimental conditions. Disulphides (Ia) undergo condensation with β-diketones[13,14] yielding enaminoketones which cyclize to 1,4-benzothiazines by scission of the sulphur–sulphur bond[1,14,15] upon attack by the nucleophilic enaminoketone systems as shown in scheme 1.

p-Bromobenzoylacetone can exist in two enolic forms **A** and **B**.

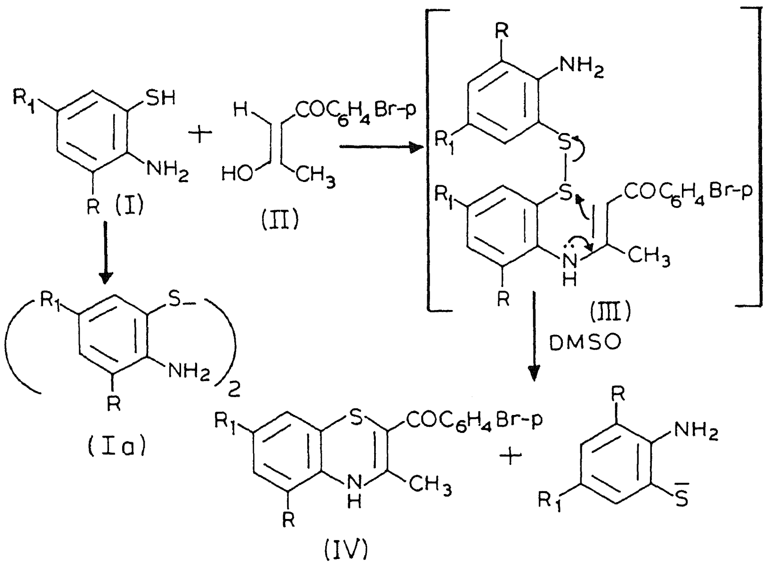


$$\underset{\displaystyle OH}{CH_3\text{–}C}=CH\text{–}\underset{\displaystyle O}{\overset{}{\underset{\|}{C}}}\text{–}C_6H_4\text{–}Br\text{-}p \qquad \textbf{A}$$



$$\underset{\displaystyle O}{\underset{\|}{CH_3\text{–}C}}\text{–}CH=\underset{\displaystyle OH}{\underset{|}{C}}\text{–}C_6H_4\text{–}Br\text{-}p \qquad \textbf{B}$$

From **A** is likely to predominate due to the electron-pushing nature of the methyl group and electron-withdrawing nature of benzene molecule, hence this form participates in the reaction.



R = H, –Cl, –CH_3, –OCH_3, –OC_2H_5
R_1 = H, –Cl, –Br, –CH_3, –OCH_3, –OC_2H_5

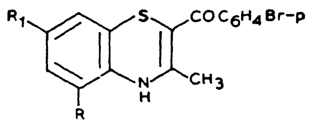
Scheme 1.

All the melting points are uncorrected and the purity of the synthesized compounds checked by TLC.

Preparation of 5- and 7-substituted 2-(p-bromobenzoyl)-3-methyl-4H-1,4-benzothiazines (IV)

A mixture of *p*-bromobenzoylacetone II (0.01 mol), 2-aminobenzenethiol I (0.01 mol) and DMSO (5 ml) was refluxed for 1 h, cooled and filtered. The product was washed with a small quantity of methanol and recrystallized from methanol. The physical and analytical data are included in table 1.

Table 1 *5- And 7-substituted 2-(p-bromobenzoyl)-3-methyl-4H-1, 4-benzothiazines (IV)*



	Compound		M.P.	Yield	Found			Mol.	Calculated		
	R	R_1		(%)	C(%)	H(%)	N(%)	formula	C(%)	H(%)	N(%)
a	Cl	H	133	40.5	50.18	2.93	3.63	$C_{16}H_{11}NSOBrCl$	50.45	2.89	3.67
b	OC_2H_5	H	109	45.5	55.26	4.13	3.61	$C_{18}H_{16}NSO_2Br$	55.38	4.10	3.58
c	OCH_3	H	158	46.0	54.16	3.75	3.78	$C_{17}H_{14}NSO_2Br$	54.25	3.72	3.72
d	CH_3	H	141	42.0	56.32	3.92	3.95	$C_{17}H_{14}NSOBr$	56.66	3.88	3.88
e	H	Br	222	68.5	45.38	2.61	3.25	$C_{16}H_{11}NSOBr_2$	45.17	2.58	3.29
f	H	Cl	241	65.0	50.62	2.93	3.71	$C_{16}H_{11}NSOBrCl$	50.45	2.89	3.67
g	H	F	221	63.5	52.32	3.06	3.81	$C_{16}H_{11}NSOBrF$	52.74	3.02	3.84
h	H	OC_2H_5	220	62.5	55.66	4.06	3.55	$C_{18}H_{16}NSO_2Br$	55.38	4.10	3.58
i	H	OCH_3	204	60.5	54.38	3.76	3.68	$C_{17}H_{14}NSO_2Br$	54.25	3.72	3.72
j	H	CH_3	211	70.0	56.28	3.83	3.92	$C_{17}H_{14}NSOBr$	56.66	3.88	3.88
k	H	H	193	55.0	55.28	3.49	4.08	$C_{16}H_{12}NSOBr$	55.49	3.46	4.04

IR (KBr): 3280–3320 cm^{-1} (NH); 1570–1610 cm^{-1} (C=O), 1350–1370 and 1450–1475 cm^{-1} (CH deformation vibrations of C–CH_3).

^{1}H NMR (DMSO-d_6): The spectra of all the synthesized compounds exhibit a signal in the region δ 8.3–10.7 due to the N–H proton, a singlet in the region δ 1.9–2.8 due to methyl protons and a multiplet in the region δ 6.2–8.17 due to aromatic protons.

Mass spectra: Mass spectra were recorded on a JEOL, JMSD-300 mass spectrometer at 70 eV and 100 μA ionizing current. All the compounds, besides the respective molecular ion peaks exhibited a peak at $m/z = 184$ due to the $(COC_6H_4Br\text{-}p)^+$. This supports the formation of benzothiazines with benzoyl group rather than acetyl group.

Financial assistance from UGC, New Delhi, is duly acknowledged. Thanks are due to RSIC, Lucknow for providing IR and mass spectra.

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ISOLATION OF A NEW TRITERPENE FROM *CALOTROPIS PROCERA* LATEX

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A variety of terpenoid alcohols, esters of steam volatile and long chain fatty acids and several cardioactive poisons have earlier been isolated from *Calotropis procera* latex and characterized. In the present communication, we describe the isolation and identification of a new ester of a terpenol.

The pooled frozen latex was thawed a fortnight after collection when the serum separated and the solid part of the latex could easily be removed and dried between filter paper sheets.

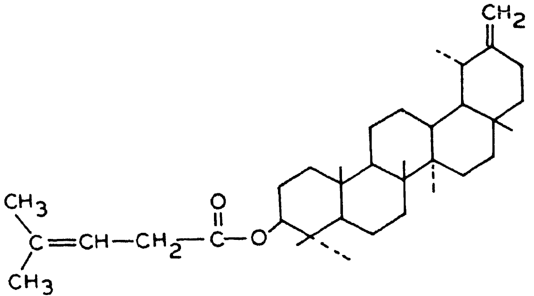
The solid was extracted exhaustively with ethanol (95%, v/v) and the alcoholic extract filtered hot and cooled when it deposited a white bulky mass. This was filtered, dried and subjected to column chromatography on a silica gel column dissolved in *n*-hexane and eluted with the same solvent.

The fractions eluted yielded two compounds. While one of them was identified as taraxasteryl acetate by various physical and chemical tests (m.p., m.m.p., IR, NMR, MS and co-TLC) the other appeared to be a new triterpene.

The white crystalline compound melting sharp at 154°C and with 522 as its molecular weight determined by the Rast's method, answered to the molecular formula $C_{36}H_{58}O_2$. (Found: C, 82.7%; H, 11.16%; $C_{36}H_{58}O_2$ requires C, 82.76%; H, 11.11%). The compound responded to all the colour tests for triterpenes.

The IR spectrum exposed strong bands at 1740 cm^{-1} ($\rangle$C=O group), 1460^{-1} cm, 1396 cm^{-1}, 1260 cm^{-1} (–O–COR group) and at 880 cm^{-1} (=CH_2 group out of plane bending).

The ^{1}H NMR spectrum of the compound revealed the presence of an exocyclic methylene group (δ 4.50, 2*H*, C=CH_2). The signals at 0.79, 0.85, 0.92, 0.95



Scheme 1.

and 0.99 (21) established the presence of seven methyl groups on a saturated carbon atom. The compound also exhibited the characteristic signals for an ester side chain comprising of 2.06 (2H, $-CH_2-$), 5.2 (t, 1H, $-CH=C\langle\,$) and 1.32 $\left(\mathrm{S}, 6H, =C\langle^{CH_3}_{CH_3}\right)$.

Hydrolysis of the compound in alkaline medium yielded a compound whose elemental composition, molecular formula, physical properties (m.p., IR, NMR and MS) and the presence of a single –OH group at C_3 position pointed it to be taraxasterol. The mixed melting point of the compound with an authentic specimen of taraxasterol did not register any depression in its melting point.

Based on the above observations, the new compound obtained has been assigned the structure (scheme 1) and named as Taraxast–20(30)en-3-(4-methyl-3-pent-enoate).

The authors are grateful to Dr F. A. Hussaini, CDRI, Lucknow, for helpful discussion and suggestions.

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BHIDEA BORII, A NEW SPECIES OF POACEAE FROM INDIA

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WHILE studying the grasses at the herbarium of the Royal Botanic Gardens, Kew, one of us (VP) found an interesting specimen collected by C. McCann during October 1919 from Siddapur (Karnataka State). Detailed studies of the specimen revealed that it is a new species of the genus *Bhidea* Bor. The same is described here.

Bhidea borii U. R. Deshpande, Ved Prakash and N. P. Singh, *sp. nov.*

Ex affinitate *B. burnsiana* Bor et *B. fischeri* Sreekumar and Shetty, ab utrogue spiculis sessilibus magnioribus (ca 2 cm longis) et spiculis utrogue glumis aristatis distinctus.

Holotypus: India: Jog–Siddapur (ditione Karnataka) *McCann* A. 51a (K).

Bhidea borii U. R. Deshpande, Ved Prakash and N. P. Singh, *sp. nov.*

Resembles *B. burnsiana* Bor and *B. fischeri* Sreekumar and Shetty (in *Kew Bull.* 42(3): 683. f. 1. 1987) but differs from both in having larger sessile spikelets *ca* 2 cm long and glumes of all spikelets with awns.

Annual, *ca* 16 cm high. Culms slender, upper nodes hairy, hairs up to 2 mm long. Leaf blade linear, acuminate, glabrous, 2.5–6.0 × 0.2 cm; sheath glabrous. Inflorescence terminal, solitary, up to 5 cm long. Spikelets in pairs, one sessile and the other pedicelled, except the lower 3 pairs at the base of the raceme, which are homomorphous. Rachilla joint, *ca* 2 mm long, hairy. Sessile spikelet *ca* 2 cm long; callus 2.5 mm long; lower glume 1.4 cm long, awned; upper glume 2 cm long, 3-lobed, midlobe long-awned; lower lemma 1 cm long; upper lemma 3.7 cm long including the awn, awn *ca* 3 cm long, geniculate. Pedicelled spikelet 1.7 cm long including pedicel; pedicel 3 mm long, hairy on one side; lower glume 1.2 cm long, lanceolate, shortly awned, margins hyaline; upper glume 1.4 cm long, lanceolate, long-awned.

India, Karnataka, North Kanara District, Jog–Siddapur *C. McCann* A51a, October, 1919 (holotype K!).

Habitat: In open grasslands on rocky soils growing along with *Danthonidium gammie* (Bhide) C. E. Hubbard.

Etymology: This species is dedicated to the memory of Dr N. L. Bor, a pioneer in the study of grasses.

The authors thank the Director, Botanical Survey of India, Calcutta, for facilities and the authorities of the Royal Botanic Gardens, Kew, for permission to consult their herbarium. They are also thankful to

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23 February 1989

A NEW SEVERE MOSAIC DISEASE OF OPIUM POPPY

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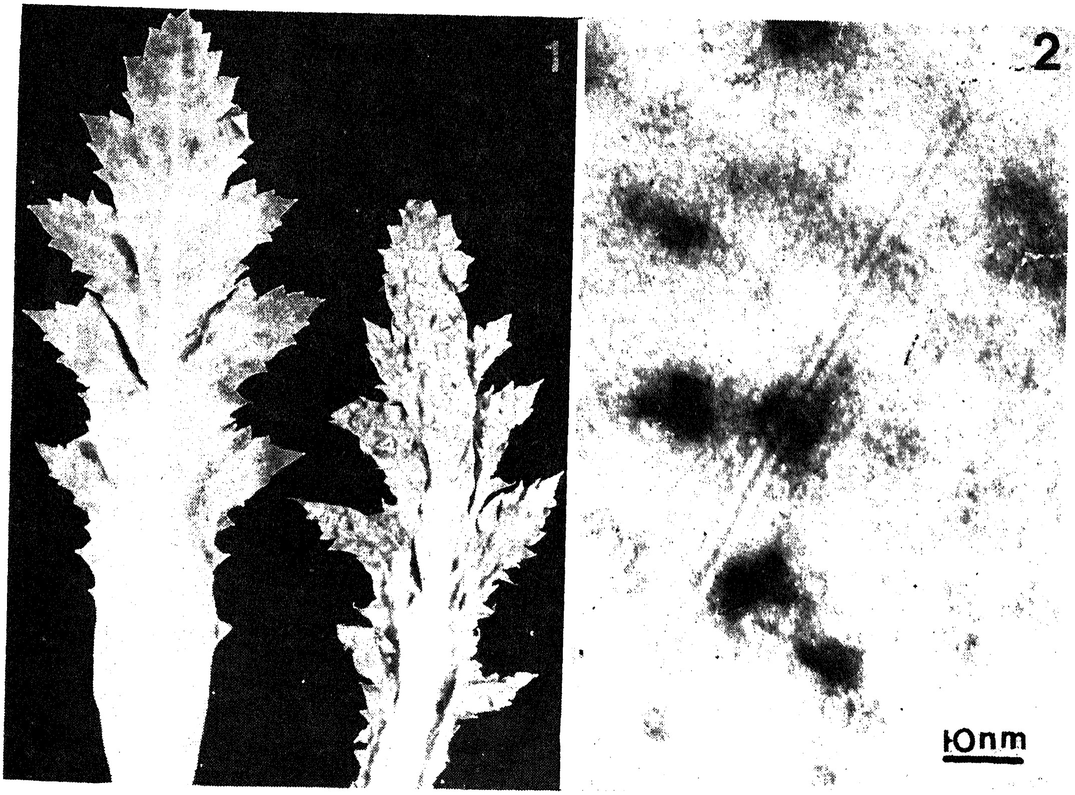
OPIUM poppy (*Papaver somniferum* L.) is an important medicinal plant cultivated on a large scale in India. It contains several alkaloids; among them morphine, codeine, thebaine, narcotine and papaverine have important therapeutic value.

A severe mosaic disease was observed on opium poppy plants, in the experimental fields of the Central Institute of Medicinal and Aromatic Plants, Lucknow. The affected plants exhibited mosaic, puckering symptoms on the leaves. Plants were stunted, and produced small flower buds with mottling symptoms on pedicel and sepals. The capsules were distorted. Severe infection of young plants often resulted in premature death of the plants.

For mechanical transmission experiments, the sap was extracted from the infected leaves in 0.1 M phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol and gently rubbed on healthy young leaves of test plants previously dusted with Carborundum (600 mesh).

Aphids (*Myzus persicae* Sulz.) were used in insect transmission tests. Virus-free insects were fed on infected *P. somniferum* plants for 24 h. After fasting them for 4 h the aphids were transferred to two sets of healthy one-and-a-half-month-old poppy plants. Twenty-four hours later, 0.03% rogor was sprayed



Figures 1 and 2. **1,** Healthy and mosaic virus-infected poppy leaves. **2,** Flexuous filamentous virus particles in leaf-dip preparation.

to kill the aphids in one set. From the other set of the plants aphids were transferred to another set of healthy plants. This process was repeated for 10 days to test for non-persistent or persistent virus transmission.

Seventy per cent of mechanically inoculated opium poppy plants showed mosaic symptoms 9–10 days after inoculation (figure 1) and veinal necrosis after 18–20 days, which led to premature death of the plants. Only 5% of the plants inoculated by insect transmission exhibited symptoms of the disease, and the virus transmission was non-persistent[1-3].

Host range of the virus was very limited. However, *Sonchus asper* L. and *Argemone mexicana* L. showed severe systemic symptoms after inoculation. *Chenopodium amaranticolor* Coste & Reyn and *C. quinoa* Willd. produced pale chlorotic lesions 5 days after inoculation. Thermal inactivation point of the virus was 50–55°C; the virus retained infectivity for up to 50 h at 30–35°C and showed pathogenicity at dilutions up to 10^{-3}.

Electron microscopic examination of leaf-dip preparations revealed that the virus is a flexible rod, about 765 nm long. Infected cells exhibited numerous scrolls and a few pin-wheel inclusion bodies. Turnip mosaic virus, a member of the potyvirus group, has been reported on opium poppy from Hungary[2]. Bean yellow mosaic virus has also been reported to naturally infect opium poppy in Bulgaria[4]. A strain of turnip mosaic virus causing mosaic disease of radish has, however, been reported to be mechanically transmitted to poppy[5]. A viral disease of opium poppy has also been reported to be caused by a spherical virus[1]. The present virus differs significantly in particle morphology[6], biological properties and symptomatology[7] from the above reports[1,7]. On the basis of host range, biological properties, insect transmission and electron microscopic studies, the present isolate appears to be a member of the potyvirus group. A perusal of the literature indicates that this is the first report of occurrence of a potyvirus on opium poppy in India[7,9].

The author thanks Dr R. S. Thakur and Dr K. K. Janardhanan for encouragement and suggestions.

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RAPID EFFECTS OF LEAD ON WATER HYACINTH (*EICHHORNIA CRASSIPES* SOLMS) PLANTS FOLLOWING SINGLE EXPOSURE DETECTED BY PHOTOACOUSTIC SPECTROSCOPY

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LEAD is a biologically nonessential metal. It is well known for its toxicity to plants and animals[1,2]. Even 10 ppm of lead in solution can stop root growth completely in certain plants[3]. It inhibits the activity of many enzymes that have key roles in cellular metabolism and in biosynthesis of some important compounds such as haem and chlorophyll[1,2,4]. Incubation of *Anacystis nidulans* thylakoids with Pb^{2+} (0.5 mM) for 30 min at 4°C removes 60% of the Mn pool. Only 0.5 mM of Pb^{2+} reduces oxygen evolution to zero[5]. Many sources of lead are responsible for contamination of the environment. Lead mining, industrial use of lead and combustion of petrol containing lead additives are the main causes of lead pollution. Urban areas and rural areas near major roads are more likely to be subjected to contamination by these sources. Plants growing in these places are highly exposed to lead, which gets deposited in various plant tissues and becomes a potential causal factor in plant pathology[1,6]. Lead taken up by plants becomes a source of lead poisoning for animals.

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†For correspondence

The present study was carried out with a view to find out the rapidity of post-exposure effects of lead on water hyacinth (*Eichhornia crassipes* Solms) plants by using the sensitive technique of photoacoustic spectroscopy. These plants were chosen for the study because they absorb metals very rapidly. They can absorb 65% of lead from the surrounding medium within an hour of exposure to 10 ppm[6].

The plants were collected from a pond in a botanical garden. A lead nitrate solution of 100 ppm with 62.5 ppm of Pb^{2+} was prepared in 16 l of pond water in a big plastic bowl. Four similar plants were kept in the solution. Four other similar plants were kept in the same amount of pond water in a second bowl as controls. Every 24 h a single plant was picked up from each of the bowls and washed with tap-water to remove lead nitrate deposited on the surface. A photoacoustic (PA) spectrum of one leaf of both exposed and control plants was taken immediately after removal without allowing them to dry. Spectra were recorded at 24, 48 and 72 h of exposure. All the spectra were taken with a modulation frequency of 80 Hz on a single beam PA spectrometer (model OAS 400, EDT Research, London) with a 300 W xenon lamp. The PA spectra were measured under identical conditions of illumination and corrected using the same carbon black reference sample.

Figure 1 shows PA spectra of a control sample and of samples taken from lead-exposed plants after 24, 48 and 72 h. A strong PA signal is seen in the wavelength range 200–400 nm whereas a weak one is seen in the range 400–800 nm. This is attributed to the presence of the cuticle, which is transparent to visible light but presents a thermal barrier to the diffusion of heat generated in the underlying material following absorption of visible light[7]. But the cuticle itself absorbs in the wavelength range between 200 and 400 nm and the heat generated following this ultraviolet absorption faces no thermal barrier.

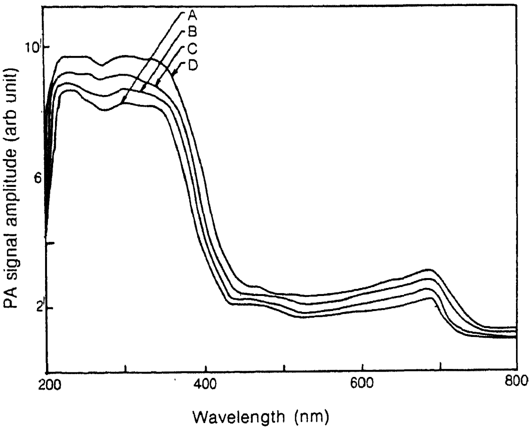

Figure 1. Photoacoustic spectra of water hyacinth leaves. A, control; B, 24 h exposure of plant to lead nitrate; C, 48 h exposure; D, 72 h exposure.

An enhancement in PA signal amplitude all along the spectral range was observed following the lead exposure, and signal amplitude increased with increase in exposure time (curves B, C and D of figure 1). Lead being a heavy cation, most of the absorbed lead gets deposited in the root system of the plant. Wolverton and McDonald[6] observed by atomic absorption spectroscopy that lead gets deposited in the ratio 71:1 in roots and stem. The large amount of lead in the roots could have impaired the entire root system, seriously affecting absorption of water. As a result, the water content of the upper parts of the exposed plants could have been reduced owing to natural dehydration, causing a reduction in specific heat and density of leaf material and thickness of the cuticle. This could have resulted in an increase in the heat diffusion through various layers of the leaf including the cuticle. Because the thermal diffusivity is defined as $\alpha = k/\rho c$, where, k is the thermal conductivity, ρ, the density of leaf material and c, the specific heat, increased diffusivity would mean accelerated heat flow through various layers and probably also partial failure of the cuticle to function as a thermal barrier. Hence an increase in PA signal was observed. Rosencwaig and Pines[8] also reported a

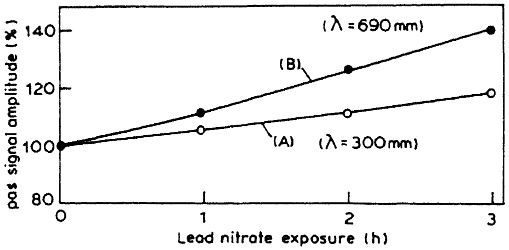

Figure 2. Ratio of signal amplitude at two wavelengths of PA spectra of leaves from lead-exposed plants to the signal amplitude of spectra of control sample as a function of duration of exposure.

similar relationship between PA signal and water content of sample in experiments with rat stratum corneum. Figure 2 shows plots of ratio of signal amplitude versus time of exposure for two wavelengths, 300 and 690 nm. It can be seen that the ratio of signal amplitude at 690 nm increases rapidly compared to that at 300 nm. This suggests that there may be effects in addition to those mentioned above. The extra enhancement of signal at 690 nm could be due to inhibition in photosynthesis caused by lead, and reduction in photosynthesis causes reduction in photochemical energy loss, making more absorbed light energy available for nonradiative relaxation[9]. Lead affects biosynthesis of chlorophylls and haem through inhibition of δ-aminolaevulinate dehydratase[2]. It still remains to be seen in what way lead exposure affects photosynthesis.

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TRATHALA FLAVO-ORBITALIS CAMERON (ICHNEUMONIDAE)—PARASITE OF *LEUCINODES ORBONALIS* GUEN. FROM BIHAR

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Leucinodes orbonalis Guen. has been reported as a serious pest of brinjal shoot and fruit throughout India[1]. Investigations were carried out to record its parasitoids for biocontrol in Bihar.

A mass collection of *L. orbonalis* was made and the insects were reared in the laboratory from August 1986 to July 1988 all over Bihar in different seasons. The parasitoids that emerged from puparia of *L. orbonalis* in the course of rearing were identified as *Trathala flavo-orbitalis* Cameron (Ichneumonidae: Cremastinae). Pupal period of parasitized *L. orbonalis* was longer, 11 to 18 days, compared to 6–14 days for normal pupae. The parasitoids are 8 ± 1 mm in length and 9.5 ± 1 mm in width across the wings. They bear a long ovipositor of 3 ± 1 mm and antennae of 5 ± 1 mm. The wings are hyaline with stigma. Colour is pale brown, and the head dark brown (figure 1). Adults are thelyotokons. Adult parasitoids lived for 4–7 days.

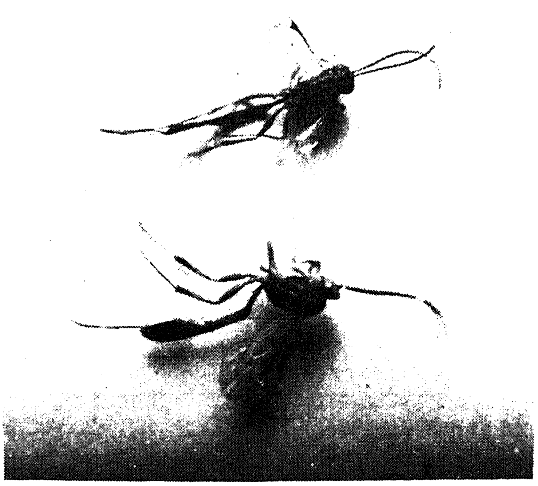

Figure 1. Adult females of *Trathala flavo-orbitalis.*

In India, this parasitoid was first reported from Maharashtra[2] and then from Gujarat[3]. Other parasitoids recorded from other parts of India, viz. *Pristomerus testaceus*[4], *Phanerotoma* sp.[5,6], *Campyloneura* sp.[5] and *Pseudoperichaeta* sp.[5,6], have not been encountered in Bihar. Further, cumulative parasitization by *Phanerotoma* sp. and *Campyloneura* sp. was of low level, 1–2%, whereas our studies on *T. flavo-orbitalis* revealed that parasitization varied between 3.57% in February and 9.06% in November. Studies are in progress to asses the usefulness of the parasitoid as a biological control agent against *L. orbonalis*.

The authors thank Dr J. R. Barron, BRI, Canada, and Dr S. I. Farooqui, IARI, New Delhi, for identifying the parasitoid, and ICAR, New Delhi, for financial assistance.

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BIOCOENOTIC ASSOCIATION BETWEEN NITROGEN-FIXING AND PHOSPHATE-SOLUBILIZING MICROORGANISMS

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INTRODUCED microorganisms can grow in a soil environment such as the rhizosphere provided the nutritional status there suits the microorganisms and the inoculum is large enough to give them an initial competitive advantage over the normal rhizosphere population. In such conditions the introduced microorganisms can affect the development of the plant. These microorganisms may disturb the soil equilibrium; but a continuous interaction exists among these organisms, and they may revert to a stabilized equilibrium. Interaction among the microorganisms is governed by several factors, viz. competition for nutrition, inhibition or acceleration of growth by other organisms, utilization or exchange of metabolites, and, sometimes, symbiotic existence. An understanding of these factors will help in better appreciation of the plant–soil–microbes coenosis and its agricultural implications, and in the development of mixed inoculants. The present study was conducted to see the biocoenotic association between nitrogen-fixing and phosphate-solubilizing microorganisms under *in vitro* conditions.

The cultures used were *Azotobacter chroococcum* (strain 2) and *Pseudomonas striata* (strain 27), obtained from the Culture Collection Centre, Division of Microbiology, IARI, New Delhi. These two organisms were first tested for antagonistic action, if any, by the 'cross streaking' method. No antagonistic effect was observed, and this was further confirmed by the 'filter paper disc' method. Solubilization of tricalcium phosphate (TCP) in Pikovskaya's broth with single and mixed culture was studied. In each case a one-ml thick suspension of bacteria (OD 1.0) was inoculated. In the treatment where both cultures were added, the total quantity of the inoculum was kept constant (1.0 ml) and the two organisms were added in equal proportions. The flasks were incubated at 30±2°C in a BOD incubator and observations were made at regular intervals. Nitrogen fixation by pure and mixed cultures of the test organisms was studied by growing the organisms in Jensen's nitrogen-free medium. Nitrogen fixed was estimated at regular intervals by the standard method[1].

The study was undertaken to assess the effect of interaction between these two organisms on their growth pattern. The results indicated that *A. chroococcum* and *P. striata* could grow together and no antagonistic behaviour of one organism towards another was noticed, stressing the feasibility of using these two together as microbial inoculants. Table 1 shows the rate of phosphate solubilization by individual and mixed cultures. Both bacteria were able to solubilize TCP to varying degrees. Maximum solubilization was observed with the mixed culture. The co-existence of the two species could be explained by the fact that nitrogenous compounds and growth-promoting substances synthesized by *Azotobacter* could be utilized by *Pseudomonas*, and

Table 1 *Phosphate solubilization by pure and mixed cultures in Pikovskaya's medium*

	Period of incubation (days)							
	7		14		21		28	
	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)	pH of medium	Soluble P (mg/100 ml)
A. chroococcum	5.82	5.7	5.96	8.0	6.00	8.7	5.86	9.6
P. striata	4.90	25.0	5.20	17.2	5.56	12.1	5.91	10.8
A. chroococcum + *P. striata*	4.81	28.9 (15.6)*	5.08	19.1 (11.0)	5.63	13.0 (7.4)	5.80	11.6 (7.4)

*Per cent increase over *P. striata* alone.

Table 2 *Nitrogen fixation by pure and mixed cultures in Jensen's medium*

	mg N fixed/g sucrose oxidized			
	Period of incubation (days)			
	15	30	45	60
A. chroococcum	20.0	19.5	16.1	12.5
P. striata	1.3	6.2	4.3	4.5
A. chroococcum + *P. striata*	17.8	17.3	15.3	11.1

Azotobacter growth was stimulated owing to the presence of available phosphorus in the culture medium. The results also indicate an inverse correlation between phosphate solubilization and pH of the medium. With the increase in pH with time of incubation, there was a decrease in phosphate solubilization[2].

Estimates of nitrogen fixation by pure and mixed cultures (table 2) revealed that *A. chroococcum* alone, fixed a fairly high amount of nitrogen in Jensen's liquid medium, ranging from 20.0 mg/g of sucrose oxidized at 15 days to 12.5 mg at 60 days. Surprisingly, association of *A. chroococcum* with *P. striata* did not improve the nitrogen-fixing capacity of *Azotobacter* and the quantity of nitrogen fixed was less than that by *A. chroococcum* alone. This is in conformity with an earlier finding[3] and could probably be due to assimilation of the fixed nitrogen by *Pseudomonas*.

Thus, synergistic interaction of *P. striata* and *A. chroococcum* in increasing phosphate solubilization may prove beneficial for developing a mixed inoculant for increasing crop productivity.

25 December 1988

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LEVELS OF SERUM ANTIOXIDANTS IN DOXORUBICIN-TREATED RATS—INFLUENCE OF VITAMINS E AND C

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DOXORUBICIN (also known as adriamycin) is a broad-spectrum chemotherapeutic agent effective against human malignancies such as leukaemias, lymphomas and many solid tumours[1]. Formation of free radicals as well as accumulation of lipid peroxides have been well documented in heart[2] and serum[3] of patients and experimental animals under doxorubicin therapy. The increased peroxidation of polyunsaturated fatty acids is recognized as one of the possible biochemical mechanisms of genesis of membrane injury in the myocardium[4]. Generally, iron in its free form is believed to accelerate lipid peroxidation induced by free radicals[5]. A major part of extracellular antioxidant defence is to keep this ionic iron largely sequestered in proteins[6]. Albumin, haemopexin and haptoglobin have been shown to inhibit various radical reactions probably by sequestering metals[6].

Since the deleterious effects produced by free radicals depend upon the balance between oxidant and antioxidant capacity of the system, we investigated the levels of the extracellular antioxidants uric acid, ceruloplasmin and albumin, and total iron-binding capacity. Serum iron and lipid peroxides were also measured and compared with rats co-administered vitamins E and C.

Doxorubicin was purchased from Sigma Chemicals, USA. Male albino rats, derived from Wistar strain, weighing 90–100 g, were selected and divided into 3 groups. The animals were allowed free access to food and water. Group I served as control. Group II and III animals were treated with doxorubicin 2 mg/kg body weight twice weekly for 4 weeks intravenously[7]. Group III animals also received vitamin E (40 mg/rat/day) and vitamin C (100 mg/rat/day). The vitamin therapy was carried out for 2 months. After the experimental period the animals were killed by cervical dislocation and the blood was collected and serum separated. Whole blood (0.5 ml) was taken for glutathione[8] estimation. Albumin[9], ceruloplasmin[10], uric acid[11], iron[12] and total iron-binding capacity[13] in serum were measured. Serum lipid peroxides were measured by the method of Yagi[14]. Vitamin E was determined by the method of Desai[15] and vitamin C by the method of Kyaw[16].

The levels of serum lipid peroxides, iron and total iron-binding capacity are presented in table 1. A significant increase in lipid peroxides and iron content is evident in group II animals, which could be attributed to the low iron-binding capacity of the serum. The increased circulating iron may have been responsible for induction of free radical formation. Vitamin-treated animals show decreased lipid peroxide, which shows the efficacy of these vitamins as antioxidants. Iron-binding capacity is also increased under vitamin therapy.

Table 2 shows the levels of ceruloplasmin, albumin and uric acid in control and experimental groups. The levels of these antioxidants decreased considerably in group II animals. Ceruloplasmin oxidizes Fe(II) to Fe(III), which swiftly binds to transferrin[17] and is ineffective in inducing free radical reactions. Uric acid and albumin[18] have also been shown to be good chelators of metal ions which promote lipid peroxidation. We have already reported low level of serum protein[19] in doxorubicin-treated rats.

Table 1 *Levels of serum lipid peroxides, iron and total iron-binding capacity in control and doxorubicin-treated albino rats*

Group	Serum lipid peroxides (nmol MDA/ml)	Serum iron (μg/100 ml)	Total iron-binding capacity (%)
Control	2.1 ± 0.05	40.5 ± 2.1	71 ± 1.2
Doxorubicin	4.0 ± 0.06**	63.2 ± 4.1*	49 ± 1.1*
Doxorubicin + vitamins E and C	1.83 ± 0.02	45.3 ± 2.3	65 ± 2.3

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.01$, **$P < 0.001$.

Table 2 *Levels of serum ceruloplasmin, uric acid and albumin in control and doxorubicin-treated albino rats*

Group	Ceruloplasmin (units/ml)	Albumin (g/dl)	uric acid (mg/dl)
Control	0.931 ± 0.056	3.56 ± 0.10	3.8 ± 0.15
Doxorubicin	0.506 ± 0.01*	2.05 ± 0.15*	2.5 ± 0.13*
Doxorubicin + vitamins E and C	1.405 ± 0.03	4.21 ± 0.13	3.6 ± 0.19

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.01$.

Table 3 *Levels of blood glutathione and serum vitamins E and C in control and doxorubicin-treated albino rats*

Group	Glutathione (mg/100 ml)	Vit. E (mg/100 ml)	Vit. C (mg/100 ml)
Control	60.1 ± 1.5	1.050 ± 0.07	1.82 ± 0.07
Doxorubicin	42.3 ± 2.1**	0.750 ± 0.01**	1.69 ± 0.03*
Doxorubicin + vitamins E and C	63.5 ± 2.9	1.490 ± 0.06	2.00 ± 0.04

Values are mean ± SD ($n = 6$ in each group).
*$P < 0.05$, **$P < 0.01$.

The levels of glutathione and vitamins E, C are given in table 3. A significant decrease in glutathione level is noted in doxorubicin-treated animals. Consumption of glutathione[20] during free radical-induced reactions has been well demonstrated. The activation of lipid peroxidation may have resulted in increased requirement of tissues for vitamin E and thereby decreased serum vitamin E content. Vitamin-treated animals show high vitamin E content. The level of vitamin C is not greatly elevated, probably due to continuous utilization for the regeneration of vitamin E[21]. The level of glutathione, which is an effective free-radical scavenger, is maintained in vitamin-treated animals.

From these results it can be concluded that doxorubicin or its metabolites tilt the balance towards auto-oxidation by reducing antioxidant levels, and vitamins E and C reverse this trend by accelerating the effects of metal chelators and also by their own antioxidant capacity.

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ANTIFERTILITY EFFECT OF *VINCA ROSEA* (LINN.) LEAF EXTRACT ON MALE ALBINO MICE—A SPERM PARAMETRIC STUDY

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Vinca rosea (Linn.) (*Catharanthus roseus*), of the family Apocynaeceae, with proven efficacy as anticarcinogenic[1], has also been shown to be antispermatogenic and antiandrogenic in rats[2,3]. A preliminary study[3] has shown that aqueous extract of leaves of this plant affects motility of rat sperm. The present paper reports the effect of aqueous extract of leaves of this plant on various sperm parameters of Swiss albino mice.

Male Swiss albino mice (8 weeks old, 30–38 g body weight), fed on standard mouse pellet diet (Hindustan Lever Ltd, Bombay) and water, were used. Fresh leaves of *V. rosea* were collected locally. Aqueous leaf extract was obtained according to Chinoy and Geetha Ranga[3]. Mice ($n=10$) in the experimental group received 10 mg equivalent of the dry leaf in 0.2 ml of water daily intraperitoneally for 15 days. Control mice ($n=10$) received physiological saline. The animals were sacrificed 24 h after the last injection by cervical dislocation and the cauda epididymides were removed. Modified Kreb's Ringer bicarbonate solution containing crystalline bovine serum albumin[4] was used as the medium for *in vitro* study of sperm. Cauda epididymis was placed in 200 μl of the buffer taken in an embryo cup. The cauda was punctured and minced using fine needles to release and disperse the sperm. Tissue debris was discarded, leaving a clear preparation for microscopic observation. Hanging drop preparations were made to assess sperm motility, relative percentage of live and dead sperm and sperm count; smears were prepared to assess relative proportions of normal and abnormal sperm[5].

V. rosea leaf extract did not affect body weight of the mice. Sperm count of the treated mice decreased significantly to 67% of that of controls. The relative percentage of motile sperm decreased on treatment.

**For correspondence

Table 1 *Effect of leaf extract of V. rosea on sperm parameters of male albino mice*

Parameter	Control	Experimental
Sperm count ($\times 10^6$/ml)	77.25 ± 9.09 (6)	52.13 ± 3.96** (6)
Percentage of motile sperm	71.67 ± 5.32 (6)	57.17 ± 8.80** (6)
Duration of motility (min)	92.83 ± 11.46 (6)	61.67 ± 5.96** (6)
Normal sperm (%)	85.33 ± 5.92 (6)	69.50 ± 5.96* (6)
Abnormal sperm (%)	14.67 ± 5.92 (6)	30.50 ± 5.96** (6)
Live sperm (%)	75.67 ± 5.57 (6)	61.00 ± 9.53* (6)
Dead sperm (%)	24.33 ± 5.57 (6)	39.00 ± 9.53* (6)

Values are mean ± SD.
$^*P < 0.05$, $^{**}P < 0.01$.

Duration of motility of the sperm of treated mice was 44% of that of controls. The relative percentage of abnormal sperm increased considerably. Among the abnormal sperm, categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation were predominant. The relative percentage of live sperm decreased significantly (table 1).

Sperm count is considered to be one of the important parameters that affect fertility. Decrease in sperm count of *V. rosea* extract-treated mice opens up a promising avenue for further studies in antifertility methods.

Sperm have two principal attributes, namely motility and fertilizing ability[6]. Motility is an important prerequisite for fertilization in the case of internally fertilizing organisms. Any negative impact on motility would seriously affect fertilizing ability. In increasing the relative percentage of abnormal sperms and decreasing the relative percentage of live sperm, *V. rosea* appears to be deleterious at the level of cauda epididymis.

The authors thank Dr C. Nainar Mohamed, Principal, Jamal Mohamed College, for laboratory facilities and Prof. K. Shahul Hamid for technical assistance.

26 December 1988; Revised 20 February 1989

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QUANTITATIVE ANALYSIS OF THIOCYANATE IN URINE BY HEAD-SPACE GAS CHROMATOGRAPHY

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Medico-Legal Institute, Bhopal 462 001, India

A widely used method for the determination of thiocyanate in biological fluids is the measurement of red colour of thiocyanate formed by adding ferric ion in acid medium. But it has its limitations because of interfering colours from phenols and keto acids, precipitation of some of the thiocyanate with protein by trichloroacetic acid[1,2], conversion of thiocyanate to cyanide[3], and interferences due to various coloured complexes formed with ferric nitrate reagent in the urine samples of persons under treatment with medicines such as desprin and certain antibiotics.

Other prevailing methods also have limitations, such as, in the case of Aldridge's[4] method of pyridine–benzidine reagent[5], interference by amino acids such as tryptophan and by proteins. Monitoring of thiocyanate in altered thiocyanate metabolism is of great significance, such as during therapy in certain cases of poisoning. Therefore an attempt was made to overcome such interferences in the determination of urine thiocyanate using gas chromatography.

Gas chromatographic (GC) assay of thiocyanate in terms of cyanide was made by taking 10 ml of diluted or undiluted urine in a closed vial (15 ml)

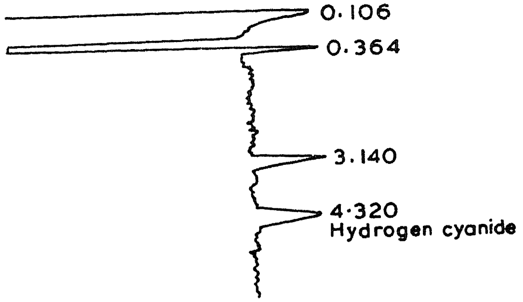

Figure 1. Chromatogram of hydrogen cyanide generated from thiocyanate in urine (head-space analysis, GC/FID).

Table 1 *Recovery of thiocyanate from urine in the GC method*

Sample no.	Amount added (mg%)	Amount found* (mg%)	Recovery (%)
1	0.1	0.090	90.0
2	0.2	0.181	90.5
3	0.5	0.460	92.0
4	1.0	0.940	94.0
5	2.0	1.860	93.0
6	4.0	3.820	95.5

*Means of three replicates.

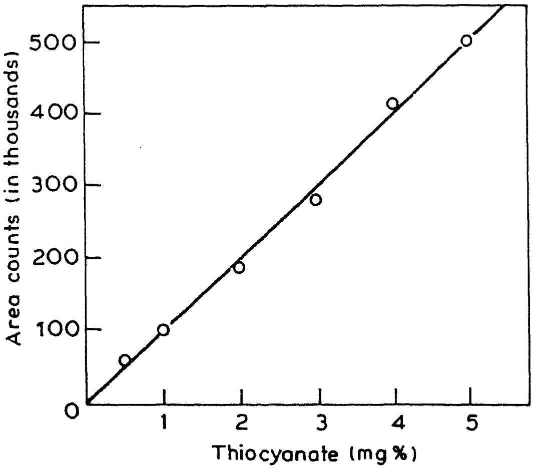

Figure 2. Correlation between Bowler's and GC methods for estimation of thiocyanate in urine.

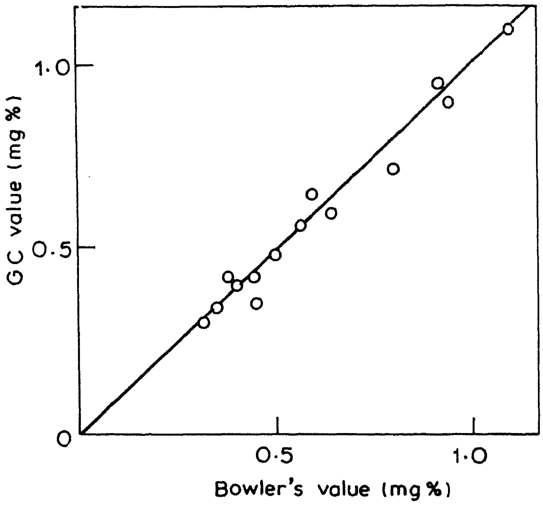

Figure 3. Standard curve for hydrogen cyanide generated from thiocyanate in urine.

sealed with Teflon tape and adding 1 ml of 0.1 M potassium dichromate and 2 ml of concentrated sulphuric acid, which converts thiocyanate to cyanide and releases it as hydrogen cyanide. Two ml of head-space gas was injected, using a pressure lock syringe (Dynatech Precision Sampling Corporation, USA) into an isothermal gas chromatograph after a 30 min incubation at 40°C, following the method of McAuley and Reive[6]. A Perkin–Elmer Sigma-300 gas chromatograph equipped with a flame ionization detector and a Teflon column (12′ × 1/8″ OD) packed with Porapak–Q 80/100 mesh was used for the analysis. Nitrogen was used as carrier gas at 45 ml/min. Column temperature was 125°C, detector temperature 230°C, and injector temperature 210°C. Recording and quantification of the peaks were done using a Perkin–Elmer LCI-100 laboratory computing integrator.

The retention time for hydrogen cyanide generated

Table 2 *Comparison of thiocyanate values of abnormal urine samples analysed by Bowler's and GC methods*

Sample	Bowler's value (mg%)	GC value (mg%)
1	2.82	0.62
2	4.50	0.14
3	2.50	0.20
4	*	0.33
5	*	0.85
6	2.80	0.50
7	1.55	0.50
8	*	1.25
9	1.65	0.52
10	1.30	0.68

*, Could not be detected.

from thiocyanate was 4.32 min and the resulting peak was uniform and symmetrical with no tailing (figure 1). Several concentrations of thiocyanate, ranging from 0.05 to 10 mg%, were used and a standard graph of area counts vs concentration was plotted; this is linear, as seen in figure 2. As the concentration of thiocyanate in the reaction mixture increased from 0.05 to 5 mg%, the variation coefficient decreased from 6.57 to 1.30% ($n = 10$ for each of the 5 test concentrations). The detector response was checked for every set of analyses by testing one or two known concentrations, allowing a tolerance limit of ± 10%. This response factor was used in analysis of samples of urine for thiocyanate levels.

Recovery experiments were carried out by adding known amounts of standard thiocyanate to urine, or cyanide to urine made alkaline. Recovery was 90–95% (table 1) and reproducibility 95–100%. Background cyanide levels in urine would not have any significant effect on the results, being in negligible amount (0.005 mg%)[7]. Phenols, keto acids, sodium thiosulphate, vitamin B_{12} and desprin did not interfere with the assay. In the case of normal urine samples no remarkable difference was noted in thiocyanate values (SD of differences was 0.041 and t was 0.91) determined spectrophotometrically using Bowler's method followed by Denson *et al.*[8] and by the present GC method (figure 3). However, abnormal urine samples, characterized either by the presence of turbidity or by a colour other than the specific red colour of ferric thiocyanate with ferric nitrate reagent, gave lower values by the present method. Urine samples in which thiocyanate could not be estimated by Bowler's method could be analysed by the GC method (table 2).

The authors thank ICMR, New Delhi, for financial assistance. They also thank Dr S. Sriramachari, Director, Institute of Pathology, and Dr P. K. Ramchandran, Director, DRDE, Gwalior, for valuable suggestions and critical discussions.

26 September 1988; Revised 27 January 1989

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ANNOUNCEMENTS

Seminar on Academy–Industry Interaction in Agriculture Biotechnology

Place: Indian Institute of Science, Bangalore
Date: 1 December 1989
Contact: The Secretary
Centre for Advancement of Biotechnology
c/o Vittal Mallya Scientific Research Foundation
K. R. Road
P.O. Box 406
Bangalore 560 004

Solid State Physics Symposium

Place: IIT, Madras
Date: 19–22 December 1989
Contact: Prof. R. Srinivasan
Convener, SSP Symposium
Dept of Physics
Indian Institute of Technology
Madras 600 036

National Seminar on Chemical Physics

Place: Pondicherry University, Pondicherry
Date: 9–11 February 1990
Contact: Prof. A. Srinivasa Rao
Chairman, NSCP
Raman School of Physics
Pondicherry University (JIPMER Campus)
Pondicherry 605 006
Abstracts by 15 December, full papers by 15 January 1990.

BOOK REVIEWS

Annual Review of Medicine, vol. 40, 1989, pp. 535, (ed.) W. P. Creger, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California 94306, USA), Price: USA $ 34, Elsewhere $ 38.

The impact of science and technology on medical concepts and practice pervades the Annual Review of Medicine for 1989. Application of the techniques of modern biology vies with the use of sophisticated instrumentation in the topics, which have been carefully selected for the volume.

Recombinant DNA technology has found important applications in medical treatment. It has produced recombinant tissue plasminogen activator (rt-PA), which is used in early thrombolytic therapy for acute myocardial infarction. Simoons has covered the extensive literature on comparative studies using intravenous streptokinase, intracoronary streptokinase, anisoylated plasminogen-activator complex (ASPAC) and rt-PA, and has reported a reduction in early mortality by 20–50%. Thrombolytic therapy is particularly effective in patients treated early. While rt-PA has not been conclusively shown to be superior to streptokinase in the reduction of mortality or bleeding complications, it has the merit of not causing anaphylactic reactions. Simoons' review contains up-to-date recommendations on the post-thrombolytic management of patients with reference to angiography, coronary angioplasty and bypass surgery. Similarly Clark *et al.* have outlined selected aspects of molecular biology in a discussion of how *bcr* and *abl* oncogenes may be involved in the pathogenesis of chronic and acute forms of Philadelphia chromosome-positive leukaemias. The use of modern biological techniques is emphasized again by Glenner, who discusses the pathobiology of Alzheimer's disease, which has assumed troublesome dimensions in the Western world. Detection of β-protein, the major component of the amyloid fibrils of cerebral vessels in Alzheimer's disease, in aged Down's syndrome individuals has been followed by the localization of the gene on chromosome 21 by the use of gene probes. It is likely that further work along these lines may improve our understanding of this disease and its management.

The best example of advanced instrumentation is the implantable cardioverter–defibrillator, which forms the subject of an excellent article by Furman and Mercando. Whereas life-threatening ventricular tachycardia is treated by medications, surgery and ablation, pacemakers and implantable defibrillators, the authors have pointed out that combining the automatic implantable cardioverter–defibrillator (AICD) and an antitachycardia pacemaker into a single device will make antitachycardia pacing an effective and more acceptable form of treatment. In another paper, Cribier and Letac have presented the current status of percutaneous balloon dilatation for the nonsurgical treatment of stenotic heart valves in children and adults. A third example of advanced instrumentation is ultrasound guidance for foetal blood sampling, which facilitates not only prenatal diagnosis of genetic disorders, infections, etc. but also foetal therapy by the administration of transfusion and drugs. Widespread use of instrumentation leading to overdiagnosis or diagnostic confusion is illustrated by Cheng in his discussion on the role of M-mode and two-dimensional echo cardiography in the detection of mitral valve prolapse.

Therapeutic advances continue to occur gradually over a wide range of diseases. Autologous bone marrow transplantation for acute leukaemias and lymphomas is an example. Santos *et al.* report disease-free survivals in 50–60% patients who fail in primary and secondary treatment regimens with this form of therapy. They have also claimed that the results of autologous bone marrow transplantation can be improved by 'purging' the marrow of tumour cells by immunological or pharmacological techniques. A more outstanding therapeutic advance is the evolution of liver transplantation as an established procedure, with over 80% survival rate at one year, thanks to cyclosporin, improved organ storage, better surgical techniques, more reliable means for detection and management of rejection episodes, and effective organization of transplant programmes (Roberts *et al.*). Advances in the understanding and management of diseases are also clearly brought out in the chapters on sarcoidosis (Johns *et al.*), non-Q-wave myocardial infarction (Gibson), chronic pancreatitis (Sarles *et al.*), prolactinomas (Molitch) and *in situ* carcinoma of the breast (Connolly *et al.*). There are also highly readable accounts on surfactants (Dobbs) and the role of neuropeptides in inflammation (Payan). The successes are however tempered by the frightening reports on cocaine dependence (over 2 million addicts), which is both psychological and neurophysiological (Garvin and Ellinwood), and lesser evils such as septic shock, which has become the commonest cause of death in intensive care units in the United States (Parrillo).

Like its predecessors, the *Annual Review of Medicine* for 1989 features articles by authorities who have made substantial contributions to their subjects and maintained a high level of seriousness in their presentations. The book is valuable for physicians and students of clinical sciences who are as keen to update their knowledge as they are to spare time and effort liberally for the task.

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Radon and its Decay Products in Indoor Air, (eds) W. Nazaroff and A. Nero, (Published by Wiley, New York,) 1988, pp. 518, Price $ 75.00.
Indoor Radon and its Hazards, (eds) D. Bodanky, M. A. Robkin and D. R. Stadler, (Published by University of Washington, P., Sealtle,) 1987, pp. 147, Price $ 9.95.
Environmental Radon, (eds) C. Richard Cothern and J. E. Smith, (Published by Plenum, New York,) 1987, pp. 363, Price $ 55.00.

Radon is practically unheard of in undergraduate physics and chemistry courses, where it is briefly introduced as the heaviest of the noble gases and as a member of the uranium series. Nevertheless, public awareness of the presence in indoor atmosphere of radon and its progeny in hazardous quantities has made radon a topic household talk. Radon gas is generated during the decay sequence of the ubiquitous, randomly distributed and naturally occurring uranium in the Earth's crust. It finds its way into indoor air through a dynamic interaction between the foundations of buildings and the soil. Once indoors, radon decays into other radionuclides. The decay of the radon progeny deposited on the lining of tissues is, on the average, the largest source of exposure to radiation the general public experiences—a surprising fact to many.

The science that has grown around the radon issue includes a variety of disciplines in physics. These are studies on the relationship between soil characteristics and radon availability, on the mechanisms of radon's entry into indoor environments, on the effectiveness of different radon mitigation efforts, and on epidemiology and the degree to which radon decay products in the air that we breathe actually pose a health risk. To date, however, this relatively young field can only measure indoor radon and cannot predict its presence; cannot relate a short-term measurement of radon to an annual average exposure; and has not completed any epidemiological study that would relate human lung cancer incidence to indoor radon exposure.

Radon and its Decay Products in Indoor Air is a well-organized compilation of technical articles covering all the major topics associated with the indoor radon problem. The chapters included in the book are divided into four foci of current research, viz. (i) sources and transport process, (ii) characteristics and behaviour of radon decay products, (iii) the basis for health concerns, and (iv) controlling indoor exposure. The topics covered contain in-depth scientific details accompanied by complete bibliographic details for the fresher. The lacuna in this book is that research on the radon problem is advancing so rapidly that some progress has been made on each topic discussed since the time the chapters were written. But the quality of the scientific content will secure the book's position as an essential starting point for any one who is interested in the radon problem for a few years to come. In a field where scientific rigour is sometimes lacking, this book is a welcome change.

Indoor Radon and its Hazards gives an overview of the radon problem. It assumes that the reader has only a little scientific background, much less than that required by the first book; for example, it begins by defining radiation and radioactivity. It uses a descriptive rather than an analytical approach to cover the same general topics as the first book, but in less detail and with less scientific rigour; thus it is not particularly useful as a scientific book. Proportionally more room in the book is devoted to dosimetry models and determining lung cancer risk due to radon exposure than to other topics. Even though a section is devoted to sources of indoor radon, the text has only one sentence on the major source, flow into the indoor air of radon-containing soil gas, driven by pressure differences between the soil gas and the indoors. The final chapter comprises a comparison of indoor radon with other radiation hazards, and nicely points out the magnitude of the problem. But this chapter could be interpreted as misleadingly endorsing nuclear power plants as safe.

Environmental Radon falls somewhere between the previously reviewed two books in content. Although this book starts, like *Indoor Radon and its Hazards*, with a basic introduction to radioactive decay, it covers the subject in more scientific detail. The main topics covered in this book are similar to those in the other two books, although, as the title suggests, the discussion does not focus in particular on radon and indoor air. Instead the book discusses a variety of topics related to radon in the environment, like radon generated from uranium mining and milling activities and from fossil fuel combustion, which are unrelated to the problems of radon and indoor air. Thus, although these are interesting topics, this book will be less useful than the other two to anyone interested in the many scientific problems associated with indoor radon. It is this specific topic that is of considerable current research interest, because of its public health aspects. In the section on sources of radon in indoor air, this book, like *Indoor Radon*, does not include a significant discussion of the dominant source, pressure-driven flow from soil gas.

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ANNOUNCEMENT

National Seminar on Recent Trends in Aquaculture

Place: Nagarjunanagar
Date: January/February 1990
Contact: Prof. Y. Radhakrishna
Centre for Aquaculture Research and Education
Nagarjuna University
Nagarjunanagar 522 510

Course on DNA Analysis in Forensic Investigation

Place: Hyderabad
Date: 1–5 January 1990
Contact: Director In-charge
Central Forensic Science Laboratory
Ramanthapur, Amberpet P.O.
Hyderabad 500 013

National Symposium on Biophysics

Place: Calcutta
Date: 20 to 22 February 1990
Contact: Prof. S. N. Chatterjee
Convener, NSB
Saha Institute of Nuclear Physics
37 Belgachia Road
Calcutta 700 037

Topics to be covered are: biocrystallography; NMR in biophysical research; IR, Raman and electronic spectroscopy applied to biomolecules; quantum biology and other theoretical aspects; computer graphics and simulation of biological systems; biomolecular interactions; radiation and photobiology; membranes; medical and clinical biophysics and related studies; analytical microscopy; microbial and cellular biophysics; and biosensors. Abstracts before 20 November, registration before 15 December.

THE SCIENTIFIC COMMUNITY

Bhatnagar Awards 1988

The Shanti Swarup Bhatnagar Awards for excellence in science and technology are given by CSIR every year to scientists below 45 years of age for outstanding research done in the country in the preceding five years. The 1988 awardees are: Prof. D. Kumar, Jawaharlal Nehru University, New Delhi, and Prof. O. N. Srivastava, Banaras Hindu University, Varanasi, in physical sciences; Dr S. K. Tandon, University of Delhi, Delhi, in earth sciences; Prof. K. Kishore, Indian Institute of Science, Bangalore, in chemical sciences; Dr B. Bhattacharya, Bose Institute, Calcutta, and Prof. M. R. S. Rao, Indian Institute of Science, in biological sciences; Dr S. Prasad, Indian Institute of Technology, New Delhi, and Prof. B. D. Kulkarni, National Chemical Laboratory, Pune, in engineering sciences.

CSIR Young Scientist Awards

The 1989 recipients of the award, given to scientists below 35 years of age for outstanding research done at a CSIR laboratory, are: Dr Jayanta Kar, National Physical Laboratory, New Delhi, and Dr V. Shubha, National Aeronautical Laboratory, Bangalore, in physical sciences; Dr Rajiv Nigam, National Institute of Oceanography, Goa, and Dr Pijush Pal Roy, Central Mining Research Station, Dhanbad, in earth sciences; Dr K. V. R. Chary, Indian Institute of Chemical Technology, Hyderabad, and Dr Sourav Pal, National Chemical Laboratory, Pune, in chemical sciences; Dr Ghanshyam Swarup, Centre for Cellular and Molecular Biology, Hyderabad, and Dr Girija Shanker Shukla, Industrial Toxicological Research Centre, Lucknow, in biological sciences; Dr Alok Kumar Saxena, Indian Institute of Petroleum, Dehra Dun, and Dr Devendra Deo Narain Singh, National Metallurgical Laboratory, Jamshedpur, in engineering sciences.

University scientist elected to New York academy

Prof. R. Nagabhushanam of the Department of Zoology, Marathwada University, Aurangabad, has been elected member of the New York Academy of Sciences. The election is in recognition of his contributions in marine biology and invertebrate endocrinology.

Patel of AT & T Bell Labs awarded IEEE Medal of Honor

Dr C. Kumar Patel, executive director of the Research, Materials Science, Engineering and Academic Affairs Division of AT and T Bell Laboratories, USA, has been awarded the Medal of Honor by the Institute of Electrical and Electronics Engineering.

Patel is widely known for his discovery of the carbon dioxide laser, which is used in industry and scientific research and is employed in medical techniques like remote probing. He is also known for his discovery of laser action on the vibrational–rotational transitions of molecules, and work in molecular spectroscopy and nonlinear optics. The latter led to the invention of the spin flip Raman lasers.

Patel, who has been with Bell Labs for over 25 years, graduated from the College of Engineering in Pune and earned MS and PhD degrees from Stanford University before joining Bell.

Patel was a recipient of IEEE's Lamme Medal in 1976. He has been honoured by many institutions, including the Optical Society of America, the Laser Institute of America, the National Academy of Engineering, the Society of Applied Spectroscopy and the American Physical Society. He is a member of the US National Academy of Sciences, IEEE, the US National Academy of Engineering and the Optical Society of America, and an honorary member of the American Society of Laser Medicine and Surgery.

Gaur moves to Delhi, Guptasarma heads NGRI

Prof. V. K. Gaur, who was Director, National Geophysical Research Institute, Hyderabad, for over six years from April 1983, was appointed Secretary to the Department of Ocean Development, Government of India, on 2 February 1989. Prof. Gaur continued to hold the post of Director, NGRI, in addition to his job at Delhi till 19 September, when Prof. D. Guptasarma assumed charge as Director.

Prof. Gaur joined the University of Roorkee as reader in geophysics in 1961 and became professor in 1966. He has made significant contributions in geophysics, particularly in seismo-tectonics and geophysical inversion theory. He was awarded the Khosla Research Prize in 1970, and the Krishnan Medal by the Indian Geophysical Union and the Shanti Swarup Bhatnagar Award in 1979. He is a Fellow of the Indian Academy of Sciences and the Indian National Science Acadmy.

Prof. D. Guptasarma, now Director, joined NGRI in 1965 as head of the Geophysical Instrumentation Division. He had worked at the Institute of Radiophysics and Electronics of Calcutta University from 1953 to 1956 and then as geophysicist in the Geological Survey of India till 1965.

Prof. Guptasarma's contributions are in radiophysics and exploration geophysics. He was awarded the Krishnan Medal in 1972 and the Decennial Award in 1986 by the Indian Geophysical Union, and the National Mineral Award for 1986–87 by the Department of Mines, Government of India. He is a Fellow of the Indian Academy of Sciences and the Indian National Science Academy.

India wins four bronze medals at Math Olympiad

India participated for the first time this year in the International Math Olympiad, held at Braunschweig, the birth place of Gauss, from 13 to 24 July 1989. The Olympiad has been held annually since 1959. India's participation this year was made possible by support from the National Board for Higher Mathematics and the Association of Mathematics Teachers of India.

The Indian team consisted of six students, picked after rigorous tests. The four who won bronze medals at IMO are Ajay Subramaniyan of Bombay, Arvind Rajaraman of Kanpur, Upendra Kulkarni of Pune and Amol S. Dighe of Thane.

More information can be had from R. Athmaraman, Admin. Secretary, AMTI, 35 Venkatesha Agraharam, Mylapore, Madras 600 004.

PROF. S. KRISHNASWAMY ENDOWMENT FUND—AN APPEAL

The reputed biologist Prof. S. Krishnaswamy (1926–1988) died after a brief illness on 20th November 1988. At the time of his death he had just spent a few weeks as Vice-Chancellor for the second term of Madurai Kamaraj University. He had a tremendous influence on teaching of and research in many branches and areas of modern biology in India. The School of Biological Sciences of the Madurai Kamaraj University, a leading centre of integrated biology teaching and research, which he built within the stifling confines of the Indian university system, is an eloquent tribute to Prof. Krishnaswamy's perseverance, dedication and vision.

A committee has been set up for the purpose of raising a 'Prof. S. Krishnaswamy Endowment Fund' to suitably commemorate the memory of one of the most self-effacing and affable scientists of our times. We appeal to all his former students, colleagues, well-wishers and friends to handsomely contribute to this Fund. Cheques/DDs may be kindly marked 'Prof. S. Krishnaswamy Endowment Fund' and then sent to the Treasurer:

Prof. T. J. Pandian
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Prof. M. K. Chandrashekaran
Secretary
Prof. S. Krishnaswamy
Endowment Fund Committee.

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COMMENT

METALLIC HYDROGEN

Introduction

Metallization of solid hydrogen at high pressures—a goal of primary importance in the field of condensed matter physics—had remained a dream for researchers working in the area of ultrahigh pressures. Recently Mao and Hemley[1] of the Geophysical Laboratory, Carnegie Institution of Washington, have succeeded in squeezing solid hydrogen to pressures in the region of 250 gigapascal (2.5 megabar). Their measurements of optical absorption and reflectivity coupled with Raman scattering provide the first direct evidence that solid hydrogen has indeed transformed to a metallic molecular solid.

The search for the insulator–metal transition in solid hydrogen has been the focus of considerable research ever since Wigner and Huntington[2] in 1935 made the theoretical prediction that at high pressures molecular hydrogen will dissociate to form atomic metallic hydrogen. Prior to this Herzfeld[3] had developed a model where the molecular polarizability is assumed to be independent of density and metallization would occur at the pressure at which the refractive index diverges.

Impetus for work in this area has also come from the speculation that atomic metallic hydrogen would be an excellent candidate for a high-temperature superconductor. An understanding of the properties of hydrogen at high pressures is also of interest to astrophysicists, for large portions of the interior of planets such as Jupiter, Saturn, Uranus and Neptune are believed to consist mostly of hydrogen subjected to pressures of several megabars. It may be mentioned here that to account for the mean density of Jupiter (which is around 1.3 g cm^{-3}), Anderson and Hubbard[4] proposed that a cubic array of neutral hydrogen atoms with sides equal to one Bohr diameter would yield a density of 1.4 g cm^{-3}. In the detailed model developed by these authors, the atmospheric layers of Jupiter consist of neutral molecular gas, and as one goes deeper, the hydrogen transforms into a molecular liquid and finally into an atomic metallic liquid (when the molecules dissociate).

Solid hydrogen

At room temperature molecular hydrogen condenses to form a transparent, electrically insulating solid when subjected to a pressure of approximately 57 kbar. In the solid state, molecular hydrogen has a number of features that distinguish it from other solids. Solid hydrogen, at normal densities, is a translational quantum solid where the molecules are freely rotating around each lattice site. The large intermolecular nearest-neighbour distance in the solid phase ($\sim$3.79 Å) and also the weak anisotropic forces lead to this interesting situation. Another characteristic feature of this quantum solid is its large compressibility (a 100% reduction in volume occurs with just 10 kbar pressure). The physical reason for this is that the lattice is expanded due to the large zero point kinetic energy. The molecules do not sit in the minimum of the attractive potential wells of the neighbours. Hence the initial compression is against this weaker 'kinetic pressure' rather than the stronger 'core pressure' one encounters in a heavier solid. Solid-state properties are also greatly affected by the presence of two distinct molecular species of hydrogen. It is well known that hydrogen consists of two molecular species, *para*-hydrogen (having the nuclear spins antiparallel and existing only in even rotational states) and *ortho*-hydrogen (where the nuclear spins are parallel and exist in only odd rotational states). Normal hydrogen is one-quarter *para* and three-quarters *ortho*. The most important difference between the two species is that, in the ground state, the *para* species ($J=0$) has a spherical charge distribution whereas the *ortho* species ($J=1$) possesses an anisotropic distribution. In the solid state, the anisotropic interaction can thus be varied by varying the concentration of the two species. Application of pressure also would enhance the anisotropic interaction.

Orientational phase transition in solid hydrogen

Apart from the exotic possibility of forming atomic metallic hydrogen at ultrahigh pressures, solid molecular hydrogen is predicted to undergo a series of interesting phase transformations under pressure[5]. The orientationally disordered *hcp* structure of the ambient pressure phase is extremely stable and persists up to nearly 1.45 Mbar pressure. Previous Raman scattering studies[6] on solid hydrogen up to 630 kbar had clearly shown that the molecular bond softens above 300 kbar pressure. Recent Raman measurements[7] by Hemley and Mao of the intramolecular stretching mode in hydrogen up to 2 Mbar demonstrate that there is a discontinuous decrease in the molecular vibron frequency near 1.45 Mbar pressure, indicative of a structural phase transformation (figure 1). It must be mentioned here that these experiments were done on solid normal hydrogen which is rich in the *ortho* species ($J=1$) and possesses a permanent electric quadrupole moment. It is believed that the electric quadrupole interactions at high densities drive this phase transition from the orientationally disordered *hcp* phase to an orientationally ordered *fcc* phase. This transformation in a pure *para*-hydrogen system is expected to occur at still higher pressures because of the lack of a permanent electric quadrupole moment for the *para* species. It is worth mentioning here that the tracking of the Raman-active intramolecular vibration mode up to 2 Mbar shows clearly that hydrogen exists as a molecular solid over this entire pressure regime.

Insulator–metal transition

Recent theoretical calculations predict that solid molecular hydrogen will dissociate to form a monatomic metal at pressures somewhere in the range 2.5 to 4 Mbar. However it is quite conceivable that, prior to this dissociation, a conducting molecular solid can be formed owing to overlap of the conduction and valence bands. Friedly and Ashcroft[8] predict such a continuous insulator–metal transition in the molecular solid with band crossing near a pressure of 2.1 Mbar.

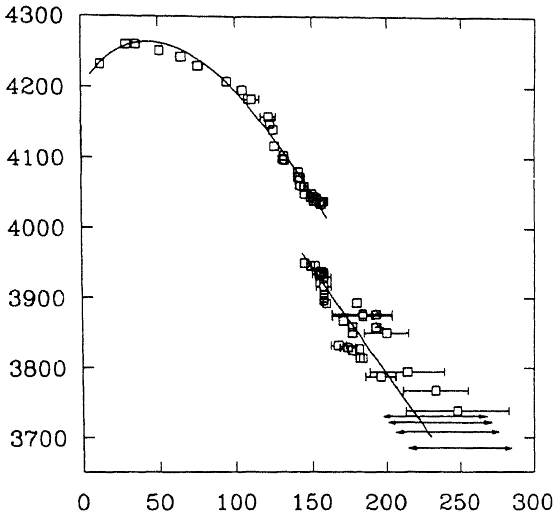

Figure 1. Pressure dependence of the molecular vibron in solid hydrogen (100 GPa = 1 Mbar). Discontinuous change in the vibron frequency near 145 GPa signifies a structural phase transition. [Data taken from ref. 7.]

Mao and Hemley carried out their pioneering work on metallization of hydrogen using a specially designed diamond anvil cell (DAC) with bevelled diamonds having central flats of roughly 25 to 100 μm dimension. It must be mentioned here that, in the year 1978, Mao and Bell at the Geophysical Laboratory had made the most significant contribution of taking the DAC to the megabar range and also extending the ruby pressure scale to this region. The main advantage of such a DAC is that, apart from generation of higher static pressures, it affords the possibility of direct visual observation and carrying out optical studies such as absorption and Raman scattering. The conduction band edge in solid hydrogen at ambient pressure is roughly at 15 eV and hence it is transparent in the visible region. If the phenomenon of pressure-induced band overlap should occur, then solid hydrogen should exhibit increased optical absorption in the visible region (1.5 to 3 eV) prior to the actual closing of the energy gap. The map of hydrogen–ruby composite sample above 2.5 Mbar pressure, along with a schematic of the diamond anvil cell, given in figure 2 (reproduced from ref. 1) clearly illustrates this increased absorption in the visible region. In the region of peak pressure, which is estimated to be in the range 2.5 to 3 Mbar, the sample is nearly opaque. Supporting evidence for the strong reduction in the energy gap with pressure also comes from the observation regarding the intensity of Raman scattering of the molecular vibron. Mao and Hemley found that the intensity of the Raman line increased ten-fold near 1.8 Mbar when the laser excitation wavelength was changed from 488 nm to 514.5 nm. This strongly suggests that electronic transitions are possible near the laser excitation energy ($\sim$2.5 eV) and the enhancement of the intensity of the Raman line can be attributed to resonance Raman scattering. Experimentation above 2 Mbar poses many challenges because of intense new fluorescence bands in diamond and measurement of pressure is rendered difficult because of the weak R_1 ruby peaks above 1.8 Mbar. Mao and Hemley[1] could not observe the molecular vibron in the Raman spectrum of the very

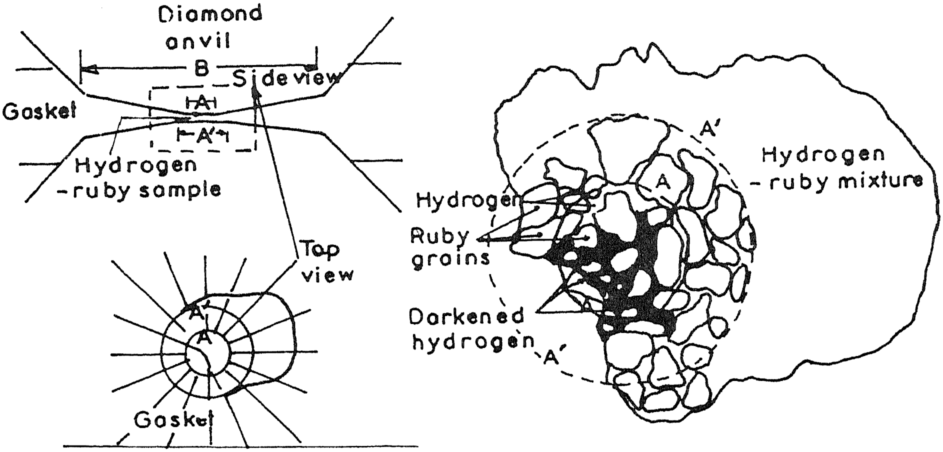

Figure 2. Map of the hydrogen–ruby sample in the small diamond flat A subjected to pressures in the region of 2.5 to 3 Mbar. Also shown is the DAC (side and top views) used by Mao and Hemley. A 25 μm, A′ 50 μm. [Reproduced from ref. 1.]

dark regions of the hydrogen sample. It is quite possible that, in these regions of ultrahigh pressures, molecular hydrogen has dissociated into atomic metallic form, although the evidence is not yet conclusive. Direct measurement of electrical conductivity in this pressure range, which would conclusively establish metallization, is a challenging problem because of the presence of a metal gasket and the small size of the anvil flat.

The electrical properties of metallic hydrogen have been theoretically investigated recently by a group led by Marvin Cohen at the University of California, Berkeley. These authors predict that, under a pressure of about 4 Mbar, metallic hydrogen would lose all electrical resistance and become superconducting with a transition temperature around 230 K. This is about 100 degrees 'warmer' than the high-T_c ceramic superconductors that have caused much excitement in the past three years. However, experimental confirmation of these interesting predictions requires new developments in the area of ultrahigh pressure generation. The experimental work of Mao and Hemley is a significant step in this direction.

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OPINION

A MODEST PROPOSAL FOR *GLASNOST* IN THE PEER REVIEW PROCESS

To my mind, one of the most important ideas to emerge in the social, political and economic history of mankind is that of **the open society**—where ideas (political and social) and products (goods and services in economics) compete in an open and free market. Each constituent in that commonwealth takes his own individual decision, exercising his own free will and his capacity for reason, and the total sum of all such decisions of the entire commonwealth of people will determine their form of government, their codes of social practice and the financial success or ruin of its small and large business and industrial enterprises.

It is this sort of openness that is still missing in our world of scientific publishing. Ideally, if the concepts of open and fair competition, the free entry and exit of ideas etc. were to be applied to our scientific community, then it would be necessary to let everyone publish and circulate his ideas in a free market of scientific enterprise, and allow the individual decisions of the scientific community add up to a vote of acceptance of the idea concerned. This is of course unreasonable and virtually impracticable—the economics of scientific publishing, strained as they are now, can never permit it. Hence the need for careful screening—the procedure we have all come to accept as the peer review process. We cannot do without it and have come to accept it as a fundamental condition, like motherhood, that one cannot argue against.

It is not really the concept of peer review that I find indefensible. It is the closed and secretive way in which it is currently practised that I find objectionable. Let us first examine how the peer review system normally works in most journals. When a manuscript reaches an editor's desk, he or an approved associate editor examines it to see if the contents are of interest to the invisible college that the journal serves, and then identifies two or three referees who can be expected to give an educated evaluation of the scientific merit of the work. When these reports come in (sometimes only one comes in, sometimes the two that come in are divided in opinion), the editor has to take an impartial decision based on his reading of these reviews to accept or reject the paper or accept after asking for revision. Throughout, the names of the reviewers remain unknown to the authors.

It is my thesis, after several unhappy experiences, that this lack of openness can lead to considerable misuse. Very often, the peers who do the review are the ones who have axioms to grind! They are the ones who have an **orthodoxy** to protect and are very quick to suppress any idea they consider **heresy**. If Copernicus or Galileo had to submit their ideas to peer review first, their efforts would never have seen the light of day. The anonymity that is now given to all peer reviewers is a licence to practice precisely this kind of scientific tyranny, giving them a mask behind which they can scrutinize and savage new ideas and stifle good and original research simply because it does not conform to the current fashions of accepted paradigms. Under such conditions, the free and fair publication of ideas leading to paradigm shift (to use a phrase made famous by T. S. Kuhn) can rarely or never take place at all. It will therefore be insurmountably difficult for **revolutionary science** to proceed at all whereas for **marginal science**, anonymous peer review is the ideal breeding ground.

The time has therefore come for some or all journals to examine this state of affairs more closely and take remedial action. Peer review should be conducted openly. The author(s) of a manuscript should not only know why their work is being turned down but also the identity of the person, or the school of thought that he subscribes to, that is turning down the manuscript. Knowing this helps to understand which school of orthodoxy has been challenged and whether there is a chance for a free trial of the ideas at all from such quarters. Stripped of this anonymity, many peer reviewers will be more careful and less likely to act out of plain malice or bias.

To reinforce my arguments, which have been impersonal so far, let me now count some of my own curious experiences in over 15 years of scientific research and after having communicated more than 60 papers to leading scientific journals. Most of my work, as would understandably be the case with most research workers, is work that can be categorized as marginal additions—very much in the nature of the **marginal science** that T. S. Kuhn has described. These are the papers I have found most easy to publish. The reviews are always kind and generous as the ideas described in such papers conform to accepted scientific paradigms and the peer reviewers are usually the same ones who have vested interests in the continued acceptance of the same paradigms. I have had considerably more difficulties with my better papers, especially where they seek to point out deficiencies in existing paradigms, and most, sometimes insurmountable difficulty with my most original ideas, especially where they directly confront and challenge the current orthodoxy and try to replace it with a new. It is instructive to scrutinize this aspect most carefully, drawing again from some of my own experiences.

I work in an area called the finite element method, a computational approach for structural analysis. I did, in 1983 to 1984, some studies on what is called the curved beam element. This is a model used to describe in numerical terms, the structural behaviour of an engineering structure like a curved arch or a circular ring. These elements were known for their notorious behaviour, if formulated strictly according to the then accepted norms of finite element practice. For about 15 years, it was believed that these finite element models did not account for what was called the *strain-free rigid-body motion of a curved element*. One could count about 30 to 60 papers (depending on what one chooses to include) representing this orthodoxy. I decided that the problem was not this at all, but something I called the need for *consistency of the membrane strain field*. Using these ideas, I believed then that I had succeeded in producing the most accurate curved beam available to date and I was also able to provide accurate error models (in a **verifiable** or **falsifiable** sense, borrowing Karl Popper's phrase) of how the inconsistent element behaved, justifying that the **consistency** paradigm had indeed a falsifiable, scientific basis. I communicated this paper to a journal. The reviews I received were contradictory. One review declared that '. . . the approach . . . appears to be very significant . . . assuming that this will be accepted in future as a fundamental contribution to the analysis of curved elements . . .'. The second review did not hesitate to dismiss the work as totally erroneous, restating the belief that it is impossible to construct the element without providing for the strain-free rigid body motion condition, and proceeded to declare that all the results given would be unpredictable and unreliable. Clearly, this peer review was protecting the territory of the old order—for the concept of consistency was heresy in the context of the old paradigm. Fortunately, in this instance, the editor was fair enough to give me the benefit of the doubt. However, I have learned that in many cases, the editors are not so bold, preferring not to transcend their usual role of a post-box.

A second incident is also instructive; both peer reviewers were agreed that the paper I wrote was not worth publishing but for diametrically opposite considerations. One reviewer declared that the work was wrong and inaccurate and was not worth considering. The other very helpfully commented that my work was obvious and a rehash of very well known ideas and that he himself had been teaching it to his students for several years. This led to a very interesting dilemma. If both reviewers had agreed that my work was false, that would have been grounds enough for outright rejection. Again, if both had agreed that it was obvious and well-known, that would have also served as grounds for summary rejection. But curiously, both recommended rejection—one, because he found my work to be new but not true and the other, because he found my work to be true but not new. Had the reviews been signed, and their identities mandatorily revealed to me, the referees would have been more careful about dismissing a piece of work in such a ridiculous way.

There are many more such incidents. I have met many who have had very similar experiences. I think, given the imperfection of the world we live in, it is understandable that this sort of situation is bound to arise. In the competitive scientific community, survival and material success is closely linked to the survival of ideas one promotes. The temptation to protect one's ideas using deliberate bias in peer review is made feasible because the perpetrator can safely hide behind the mask of anonymity that the journals provide him. I am convinced that peer review will be more effective if the reviews are all signed by the reviewers so that the defenders of orthodoxy can always be seen by the proposers of heresy. My arguments here will be incomplete if I do not include the following incident. In all my 15 years of work, I know of one reviewer who has always insisted on signing his name on the review. I have always admired that singular and courageous gentleman and I must declare that I have never had a better or more useful review from any one of the other reviewers so far.

Therefore, to conclude this modest proposal, let me make this recommendation to *Current Science*.

Let it be the first (?) journal in the world to adopt as its professed policy, the principle of an *open review system*. It need keep in its stable of reviewers, only those who understand and accept this principle—that their reviews will be signed and that their identities will be made known to the authors submitting the paper. It should also make it clear to authors that it will follow this procedure. Alternatively, it can allow authors to opt for a signed review or an unsigned review and these wishes can then be communicated to the reviewers and the process completed accordingly.

Openness, or *glasnost*, as the Russians have now come to know it, is a very noble principle—at the heart of it is the principle that justice must not only be done but must be seen to be done. I am sure many of your readers will like to comment on this suggestion. They may point out many obvious shortcomings even in an open system or may be because it is open. We will all benefit by an open debate on this in the pages of your journal.

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ANNOUNCEMENTS

New Developments in the Understanding and Treatment of Schizophrenia

Date: 6 December 1989
Place: Royal College of Physicians, London

Neuropharmacology relating to schizophrenia has progressed rapidly in recent years. For the first time since the development of neuroleptics in the 1950s, an antipsychotic with improved efficacy is available, and ongoing research promises to yield a range of new products with fewer side-effects and additional activity. This conference will bring together experts in the field to assess the progress that has been made to date, and to discuss what prospects the future holds.

New Pharmacological Approaches to Depression and Anxiety

Date: 7–8 December 1989
Place: Royal College of Physicians, London

Depression and anxiety are the two most common mental disorders, and while drug treatment for both conditions is available, it is not always satisfactory. This conference aims to present an overview of research into both conditions, outlining difficulties encountered and the limitations of current therapy. It will then examine in detail the new approaches to drug treatment now under investigation, outlining the potential advantages and reporting the latest results available for new antidepressant and anxiolytic agents.

Drugs Affecting Calcium Ions—Their Role in Modern Medicine

Date: 13–14 December 1989
Place: Royal College of Physicians, London

Calcium is a fairly ubiquitous ion found in cells and tissues that is thought to be involved in a wide range of physiological processes. It is therefore not surprising that abnormalities in the calcium system have been implicated in the pathophysiology of various disease states including cardiovascular, neurological and inflammatory. This meeting will provide a concise overview of the current states of calcium in the physiological control of cell activation and the current use of drugs affecting calcium in the treatment of various disease states. Furthermore, the meeting will review new areas of interest where drugs affecting calcium may be implicated.

For details contact: Renata Duke, IBC Technical Services Ltd., Bath House (3rd Floor),
56 Holborn Viaduct, London EC1A 2EX, UK.

Current Science, October 20, 1989, Vol. 58, No. 20

ARTICLES

The meaning of the phrase 'fundamental constituents of matter' keeps changing with time as one probes phenomena at shorter and shorter distances, hence at higher and higher energies. At the present time, this phrase applies to the quarks and leptons: the former rather elusive and only detectable indirectly, the latter more readily visible. In addition one has photons, W and Z mesons, gluons—the 'carriers' of various fundamental interactions. In this review, Prof. D. P. Roy discusses in a crisp and informative manner the prospects of and methods for detecting the sixth member of the family of quarks, the 'top' or 'truth'.

—*N. Mukunda*

TOP QUARK SEARCH*

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There are plenty of ups and downs;
And many a strange ones too.
Charm and beauty are there, though rare;
But we are yet to see the truth.

ABSTRACT

The article reviews our progress in the search for the truth (or top) quark—the last and heaviest member of the family of basic constituents of matter.

INTRODUCTION

As per our current wisdom the basic constituents of matter are a dozen of fermions: the six leptons—electron, muon, tau and their associated neutrinos; and the six quarks—up, down, strange, charm, beauty and truth (or more colloquially bottom and top) quarks. They can be arranged as three pairs or generations of leptons and quarks in increasing order of mass, as shown in table 1. The six quark types are usually called flavours.

Each pair represents two charge states differing by 1 unit of e. In addition, the quarks possess a new type of charge—the so called colour charge. All of these fundamental particles have been experimentally detected by now except for the last and the heaviest one, i.e. the top quark. Naturally top quark search is a high priority area of current and proposed particle physics experiments. The aim of this article is to give a simple overview of the top search programme—its present status and future prospects. To facilitate this discussion and fix the notations, it is useful to briefly review the basic interactions between these fundamental particles.

Apart from gravitation, which is too weak to be of interest to our discussion of subatomic particles, there are 3 basic interactions—strong, electromagnetic and weak. They are all gauge interactions mediated by vector particles. The strong interaction is mediated by the exchange of massless vector gluons, which couple to all coloured particles (quarks) with coupling proportional to the colour charge C (figure 1a). This is analogous to the electromagnetic interaction, mediated by exchange of

Table 1

Leptons			Charge	Quarks			Charge
ν_e	ν_μ	ν_τ	0	u	c	t	2/3
e	μ	τ	−1	d	s	b	−1/3

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*This article is dedicated to my teacher and one of the leading contributors to the quark model, Prof. A. N. Mitra, on the occasion of his sixtieth birthday.

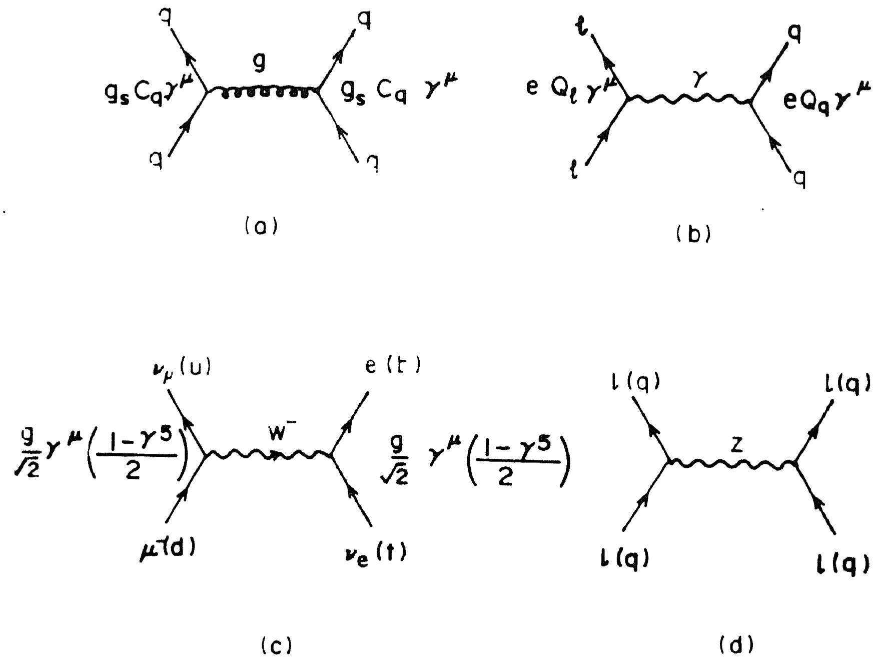

Figure 1. Basic interactions of quarks and leptons. **a,** Strong. **b,** Electromagnetic. **c,** Charged current weak. **d,** Neutral current weak.

massless vector photon, which couples to all charged particles (quarks and charged leptons) with coupling proportional to the electric charge Q (figure 1b). It is more customary to write the strong coupling as

$$\alpha_s = g_s^2/4\pi \qquad (1)$$

in analogy with the fine structure constant

$$\alpha = e^2/4\pi. \qquad (2)$$

The weak interactions are mediated by the massive charged and neutral vector bosons $W^\pm$ and Z^0. The charged $W^\pm$ boson couples to each of the above pairs of leptons and quarks with the same universal coupling g (figure 1c), where the combination of the Dirac γ matrices correspond to the famous V–A form of the charged current weak interaction giving rise to maximal parity violation. The neutral Z^0 boson couples to each quark and lepton (figure 1d), with couplings specified by the standard electro-weak model of Glashow, Weinberg and Salam. Here the weak and electromagnetic interactions are unified into a $SU(2)_L \times U(1)$ gauge interaction*, mediated by a charge triplet of gauge bosons $W^{\pm,0}$ with couplings proportional to the three $SU(2)$ generators $T^{\pm,3}$ (weak isospin) and a charge singlet B^0 with coupling proportional to the $U(1)$ generator (weak hypercharge). The two neutral bosons get mixed to give the physical Z boson and photon. It is customary to use the $SU(2)$ coupling g and the mixing angle θ_W as the 2 independent coupling parameters. Then the physical Z coupling is given by[1]

$$\frac{g}{\cos\theta_W}\left[T^3\gamma^\mu\frac{(1-\gamma^5)}{2}-\sin^2\theta_W Q\gamma^\mu\right], \qquad (3)$$

and the physical photon coupling is related to these parameters by

$$e = g\sin\theta_W. \qquad (4)$$

The quark and lepton pairs mentioned above are simply the weak isospin doublets with T^3 values $\frac{1}{2}$ and $-\frac{1}{2}$ for the upper and lower members.

INDIRECT EVIDENCE FOR TOP QUARK

There are two independent evidences for existence of a top quark as the weak isospin partner of the observed bottom quark.

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*The subscript L refers to the left handed (V–A) form of the weak interaction.

Forward–backward asymmetry in $e^+e^- \to \bar{b}b$

The axial·axial (AA) part of the Z exchange contribution to this process (figure 1d) interferes with the γ exchange (figure 1b) to give a forward–backward asymmetry in the produced b quark (see eqs. 3 & 4)[2],

$$\frac{\sigma(\theta<\pi/2)-\sigma(\theta>\pi/2)}{\sigma(\theta<\pi/2)+\sigma(\theta>\pi/2)} \simeq \frac{(-3/8)\,T_e^3\,T_b^3\,s/M_Z^2}{\sin^2\theta_W\cos^2\theta_W\cdot Q_b(s/M_Z^2-1)}. \qquad (5)$$

With $T_e^3=T_b^3=-1/2$, $Q_b=-\frac{1}{3}$ and the experimental values of the Z mass and mixing angle (from neutrino scattering experiment)[3]

$$M_Z=92.4\pm 1.8\ \text{GeV}, \quad \sin^2\theta_W=0.233\pm 0.006 \ (6)$$

one predicts an asymmetry value of about -0.25 at a centre of mass energy $\sqrt{s}\simeq 35$ GeV. The JADE collaboration has indeed observed a $25\pm 6.5\%$ asymmetry with the right sign at this energy at the PETRA e^+e^- collider[4]. In the absence of a top quark, the bottom would be a weak isosinglet ($T_b^3=0$) resulting in zero asymmetry. Thus the JADE data constitute a respectable 4σ effect, suggesting the presence of a top quark[5].

Absence of the flavour changing neutral current decay $b \xrightarrow{Z} s, d$

The eigenstates of weak isospin given in table 1 are actually not pure quark flavour states but contain small admixtures of the other two flavours, i.e. they are

$$\begin{pmatrix} d' \\ s' \\ b' \end{pmatrix} = \begin{pmatrix} V_{ud} & V_{us} & V_{ub} \\ V_{cd} & V_{cs} & V_{cb} \\ V_{td} & V_{ts} & V_{tb} \end{pmatrix} \begin{pmatrix} d \\ s \\ b \end{pmatrix} \qquad (7)$$

where V is a unitary matrix with small but nonzero off-diagonal elements (the Kobayashi–Maskawa matrix)[1]. Thus the lighter quark of a generation can decay into a lower generation by charged current weak interaction, i.e. $b \xrightarrow{W} c$, u and $s \xrightarrow{W} u$ with couplings proportional to V_{cb}, V_{ub} and V_{us} respectively. In contrast, the flavour changing neutral current couplings cancel out by unitarity of V and the universality of Z coupling to the 3 generations, i.e.

$$Z(\bar{d}'d'+\bar{s}'s'+\bar{b}'b')=Z(\bar{d}d+\bar{s}s+\bar{b}b). \qquad (8)$$

This cancellation mechanism was first used by Glashow *et al.*[6] in the context of a 2-generation quark mixing to show that the absence of the stangeness changing neutral current decay $s \xrightarrow{Z} d$ implied the existence of a c quark as the weak isospin partner of s. Similarly the absence of flavour changing neutral current decays $b \xrightarrow{Z} s, d$ suggests the existence of a t quark as the weak isospin partner of b[7]. Otherwise b' would be a $SU(2)$ singlet so that the $SU(2)$ part of its coupling to Z would vanish. The result is formally equivalent to transfering the universal $SU(2)$ coupling term $Z\bar{b}'b'$ to the right hand side of eq. (8). This would imply flavour changing neutral current decays $b \xrightarrow{Z} s, d$ with couplings $Z\bar{b}s$, $Z\bar{b}d \propto V_{tb}V_{ts}$, $V_{tb}V_{td}$. And since the diagonal element $V_{tb}\simeq 1$ and

$$|V_{ts}|^2+|V_{td}|^2=|V_{cb}|^2+|V_{ub}|^2 \qquad (9)$$

by unitarity, the total rate for the neutral current decay $b \xrightarrow{Z} s, d$ should be comparable to that for the charged current decays $b \xrightarrow{W} c$, u. Multiplying this by the $Z\to\mu^+\mu^-$ branching ratio of 3%, one predicts a $\geqslant 1\%$ branching ratio for the neutral current decay $b\to\mu^+\mu^- X$. The experimental upper limit for this decay branching ratio from the CLEO collaboration[8] is 0.12%. Thus the absence of neutral current b decay at a level comparable to its charged current decay provides a second evidence for the existence of t.

INDIRECT CONSTRAINTS ON TOP QUARK MASS

There are at least two indirect constraints on top quark mass as we see below.

Radiative correction to W & Z boson mass

It provides a clean upper bound on the top quark mass, assuming the standard model of electro-weak interaction[3, 9]. The observed rate of muon decay $\mu \xrightarrow{W} \nu_\mu e \bar{\nu}_e$ (figure 1c) determines the Fermi coupling

$$G_F=\frac{g^2\sqrt{2}}{8M_W^2}=\frac{\pi\alpha}{\sqrt{2}\cdot\sin^2\theta_W\cdot M_W^2} = 1.1663\times 10^{-5}\ \text{GeV}^{-2}. \qquad (10)$$

This gives an estimate of W mass by putting in the experimental value of $\sin^2\theta_W$ (eq. 6) and α. For a precise estimate of course one must take account of the radiative correction to the fine structure constant α. The dominant correction, coming from the photon

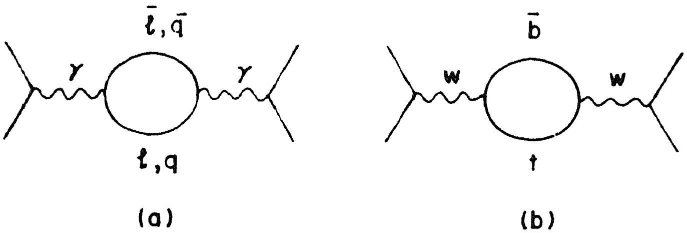

Figure 2. **a,** Lepton and quark pair contributions to photon self-energy. **b,** $t\bar{b}$ contribution to W self-energy.

self-energy diagram (figure 2a), makes α run with the mass scale, i.e.

$$\alpha(M_W^2)=\alpha(m_e^2)\left[1+\frac{\alpha(m_e^2)}{3\pi}\left\{\sum_l Q_l^2 \ln\frac{M_W^2}{m_l^2}+3\sum_q Q_q^2 \ln\frac{M_W^2}{m_q^2}\right\}\right] \quad (11)$$

to leading order in α. The summation runs over the charged leptons e, μ, τ and the light quarks u, d, s, c, b; and the factor 3 corresponds to the 3 colour states of a quark. The radiative correction factor can be easily calculated to be about 7%, i.e.

$$\alpha(M_W^2)=\alpha(m_e^2)\,[1+\Delta r] \simeq (1/137)(1+0.07)\simeq 1/128. \quad (12)$$

Since the electro-weak couplings are related at the mass scale of M_W^2, one has to use this value of α in eq. (10) to predict M_W. It gives

$$M_W^{\mathrm{Th}}=80\pm 1.5\ \mathrm{GeV} \quad (13)$$

in agreement with the experimental value of

$$M_W^{\mathrm{Exp}}=81\pm 1.3\ \mathrm{GeV}. \quad (14)$$

One should note that, without this radiative correction the predicted value is

$$M_W^0\simeq 77.2\pm 1.5, \quad (15)$$

i.e. on the margin of disagreement with data. One has a similar results for Z mass as well.

What about the radiative correction to the remaining factors of eq. (10)? It turns out to be negligible provided top quark is not heavier than W boson. For $m_t > M_W$, however, there is a significant contribution coming from the W self-energy diagram of figure 2b and the corresponding one for Z. The reason is that the longitudinal W, Z boson couplings are proportional to the fermion mass like the Higgs coupling, since the W, Z mass and longitudinal components arise by absorbing Higgs particles. Consequently the radiative correction has a quadratic M_t dependence $\left(\simeq -\frac{\cos^2\theta_W}{\sin^2\theta_W}\cdot\frac{3\sqrt{2}G_F}{16\pi^2}\cdot m_t^2 \text{ for large } m_t\right)$*. For $m_t\simeq 200$ GeV it cancels out the 7% radiative correction in eq. (12). Thus

$$m_t > 200\ \mathrm{GeV} \Rightarrow \Delta r < 0 \Rightarrow M_W^{\mathrm{Th}} < 77\ \mathrm{GeV} \quad (16)$$

in conflict with the experimental value of eq. (14). This gives an indirect upper bound on top quark mass of about 200 GeV.

$B_d - \bar{B}_d$ Mixing

There is a lower bound on top quark mass coming from the observed $B_d - \bar{B}_d$ mixing, i.e. transition between the B_d meson (bound state of the quark-antiquark pair $b\bar{d}$) and its antiparticle $\bar{B}_d$. This is a second order weak process, occurring via u, c and t quark exchanges (figure 3). If t quark was

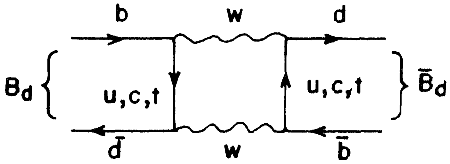

Figure 3. The second order weak process responsible for $B_d - \bar{B}_d$ mixing. There is a second diagram corresponding to the interchange of the internal quark and W boson lines.

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*Due to the W, Z mass shifts from the self-energy diagrams the standard model mass formula $\sin^2\theta_W = 1 - M_W^2/M_Z^2$ (used to estimate the $\sin^2\theta_W$ of eq. 6) acquires a radiative correction term $\propto m_t^2$. Adding this in the denominator of eq. (10) the result follows.

light like u and c, the GIM cancellation mechanism (unitarity of the V matrix) would effectively suppress this diagram. For a heavy t quark, however, there is no effective cancellation. Even more importantly, the longitudinal W contribution grows quadratically with m_t, since its coupling is proportional to the quark mass. This leads to a mixing parameter (off-diagonal element in the $B_d - \bar{B}_d$ mass matrix)[10]

$$M_{12} \simeq G_F^2 f^2 B\, m_{Bd}\, [(V_{td}^* V_{tb})^2 m_t^2 + \ldots]/12\pi^2 \qquad (17)$$

where higher order terms in m_t^2/M_W^2 are not shown explicitly. The factor $f^2 B$ arises from the strong interaction dynamics of quark-antiquark ($b\bar{d}$) binding inside the B_d meson[11]. There is no unambiguous estimate for this quantity; but various model estimates suggest the range[10]

$$f^2 B \simeq (0.10 - 0.16 \text{ GeV})^2. \qquad (18)$$

The diagonal matrix element $V_{tb} \simeq 1$, and one gets an upper bound on V_{td} from unitarity and the knowledge of the other off-diagonal elements, i.e.

$$V_{td} \leqslant 0.02. \qquad (19)$$

Thus the observation of a sizable $B_d - \bar{B}_d$ mixing would give a significant lower bound on top quark mass. The ARGUS group[12] has observed such a mixing with rate

$$r = \frac{(Re\, M_{12})^2}{\Gamma^2/2 + (Re\, M_{12})^2} = 0.2 \pm 0.1,$$

$$\Gamma^{-1} = \tau_{Bd} \simeq 10^{-12} \text{ sec}; \qquad (20)$$

where r corresponds to the probability of finding $B_d B_d$ and $\bar{B}_d \bar{B}_d$ final states in e^+e^- collision relative to $B_d \bar{B}_d$. This result has more recently been confirmed by the CLEO group. Taking the lower limit of r (eq. 20) and the upper limit of $f^2 B$ (eq. 18) gives a conservative lower bound

$$m_t \geqslant 40 - 50 \text{ GeV}. \qquad (21)$$

One should bear in mind of course that the second input is not unambiguous.

Ratio of W and Z widths

The ratio of W and Z boson widths provides an indirect probe of top quark mass up to about 60 GeV. It is simple to see this for the interesting t mass range of 40–60 GeV, in which case the $Z \to t\bar{t}$ channel is kinematically forbidden but the $W \to b\bar{t}$ channel is open, i.e.

$$W^- \to e\bar{\nu} + \mu\bar{\nu} + \tau\bar{\nu} + 3d\bar{u} + 3s\bar{c} + 3b\bar{t}. \qquad (22)$$

The opening of the $b\bar{t}$ channel would naively correspond to a 30% enhancement of Γ_W (or decrease of the ratio Γ_Z/Γ_W); but the kinematic supression factor for $m_t = 40$–60 GeV reduces it to a 15% effect. In fact the ratio does not decrease any further with m_t, since the opening of $Z \to t\bar{t}$ width compensates the further increase of $W \to b\bar{t}$ width. Thus a measurement of this ratio would tell whether there is a t quark below 60 GeV, provided the experimental accuracy is better than 15%. The ratio has been indirectly measured from the relative number of $W \to e\nu$ and $Z \to e^+e^-$ events at the CERN antiproton–proton collider (figure 4), i.e.

$$\frac{\# W \to e\bar{\nu}}{\# Z \to e^+e^-} = \frac{\sigma_W}{\sigma_Z} \cdot \frac{\Gamma(W \to e\bar{\nu})}{\Gamma(Z \to e^+e^-)} \cdot \frac{\Gamma_Z}{\Gamma_W}. \qquad (23)$$

The resulting Γ_Z/Γ_W ratio, taken at its face value, appears to favour $m_t < 60$ GeV[13]; but the errors are much too large to draw any meaningful conclusion. There is a 15% statistical error coming from the limited number of $Z \to e^+e^-$ events (~ 50) and a systematic error of similar magnitude from the estimated ratio of W and Z cross-sections[14]. The latter arises from the uncertainty of u and d quark flux distributions inside the proton, used in the cross-section estimates (figure 4). Hence it does not give any meaningful constraint on top quark mass so far.

DIRECT SEARCH FOR TOP QUARK

The progress and prospect of direct top quark search in different colliders are discussed below.

Electron–positron collider

The e^+e^- colliders provide the cleanest probe for t quark; but unfortunately the energies are too low. The simplest way to look for $e^+e^- \to t\bar{t}$ (figure 1b) is

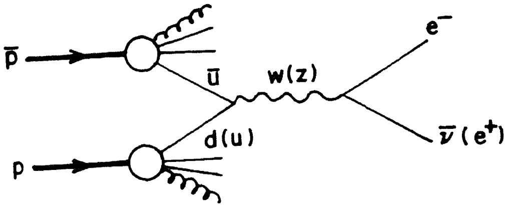

Figure 4. Production of $W \to e^-\bar{\nu}(Z \to e^-e^+)$ events in antiproton–proton collision.

through the ratio

$$R=\frac{\sigma(e^+e^-\to\text{hadrons})}{\sigma(e^+e^-\to\mu^+\mu^-)}$$
$$=\frac{\sigma(e^+e^-\to\Sigma\bar{q}q)}{\sigma(e^+e^-\to\mu^+\mu^-)}\simeq 3\sum Q_q^2 \qquad (24)$$

which should show a jump of $\Delta R=3Q_t^2=4/3$ units across the $\bar{t}t$ threshold. The second way is to look at the event shape. The lighter quark pairs fly off back to back carrying the total centre of mass energy and thus give highly collinear events. In contrast, near the $t\bar{t}$ threshold, this heavy quark pair will be produced practically at rest. And each will decay into 3 quarks (figure 1c).

$$t\to b\,u\,\bar{d},\, b\,c\,\bar{s}. \qquad (25)$$

Thus the total centre of mass energy would be shared amongst 6 light quarks, giving rise to more spherical (isotropic) events.

The PETRA and more recently TRISTAN colliders have searched for $\bar{t}t$ production using both these methods and found none. Thus they give lower mass bounds equal to their respective beam energies. TRISTAN gives the larger bound[15]

$$m_t\geqslant 26\,\text{GeV}, \qquad (26)$$

which is evidently not large enough. The LEP collider, starting later in this year, can probe top quark mass up to its beam energy of about 50 GeV. As we have already seen in eq. (21), however, it is likely to be a barren region. The LEP-II beam, scheduled for the mid-ninties, can probe top quark mass up to nearly 100 GeV. This mass range is already being probed by the current antiproton–proton colliders, as we shall see below. If they find a top quark in this mass range, then LEP-II will be very useful for a detailed investigation of its properties.

Antiproton–proton collider

The $\bar{p}p$ collider can probe a larger mass range of top quark because of its higher energy. But the signal is dirty; and one has to use special tricks to clean it up. The dominant mechanisms for top quark production are the Bethe–Heitler process of gluon–gluon fusion (figure 5)

$$gg\to\bar{t}t \qquad (27)$$

and via W (figure 4)

$$\bar{q}q\to W\to\bar{t}b. \qquad (28)$$

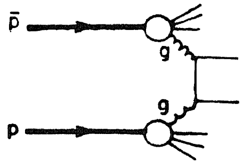

Figure 5. Top quark production in $\bar{p}p$ collisio the Bethe–Heitler process of gluon–gluon fusior

The best way to look for top is to look fo charged lepton (e or μ) coming from its lep decay (figure 1c)

$$t\to bve^+(\mu^+),\ \bar{t}\to\bar{b}\bar{\nu}e^-(\mu^-),$$

which eliminates the background from gluon ordinary quark (u, d, s) scattering. Of course charged lepton could come from the rema (unstable) quarks b and c, e.g.

$$b\to c\bar{\nu}e^-(\mu^-),\ \bar{c}\to\bar{s}\bar{\nu}e^-(\mu^-).$$

These background can be effectively suppresse requiring the charged lepton to be isolated fro other particles. Because of the large energy relea the massive top quark decay, the decay proc come out wide apart. In contrast the energy re in the light b or c quark decay is small, so tha decay products come together in a narrow con the charged lepton appears as part of the d quark jet. The isolated electron (or muon) provi simple but very powerful signature for top q first suggested in ref. 16.

Top quark search has been carried out at CERN $\bar{p}p$ collider ($S\bar{P}PS$), at a centre of mass en of 630 GeV, using the isolated electron (m signature. And the absence of such events giv lower mass limit[17]

$$m_t\geqslant 40\,\text{GeV}.$$

This is the best mass limit from direct top q search so far, and similar to the indirect limit

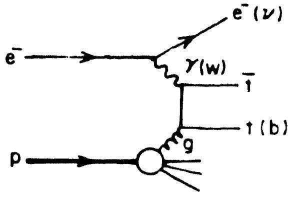

Figure 6. Top quark production in ep collision the Bethe–Heitler processes of photon–gluon an boson–gluon fusion.

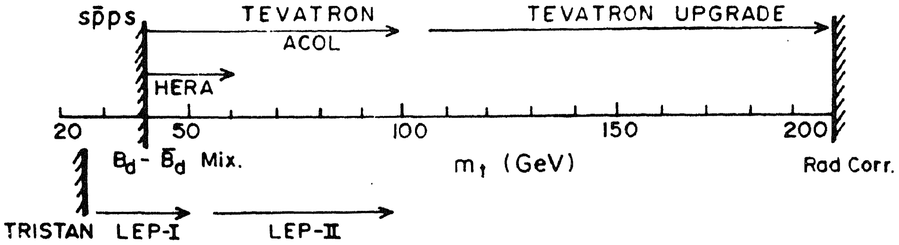

Figure 7. Top quark mass limits from the present collider experiments and discovery limits of the future ones. The indirect mass limits from $B_d - \bar{B}_d$ mixing and radiative correction to W, Z boson masses are also shown on the mass axis.

$B_d - \bar{B}_d$ mixing (eq. 21). Two top search experiments are now in progress—(i) at the Tevatron $\bar{p}p$ collider having a higher centre of mass energy of 2 TeV; and (ii) at the upgraded CERN collider (ACOL), which has an order of magnitude higher luminosity now. They are expected to probe top quark mass up to nearly 100 GeV[18]. Thus one expects to see the top quark within this year if its mass is within 100 GeV. If not then the search has to be extended to the upper mass limit of 200 GeV (eq. 16). This would be possible at the upgraded Tevatron collider[19], scheduled for the mid-ninties. Finally the superconducting super collider (SSC), expected to come at the turn of the century, can probe quark mass up to 500 GeV.

Electron–proton collider

The HERA *ep* collider is scheduled to start operation in 1990. Unfortunately the *ep* collider does not provide as clean a top quark signal as the e^+e^- collider, nor does it have the mass reach of the $\bar{p}p$ machine. The dominant top quark production mechanisms are the Bethe–Heitler processes of photon–gluon and W boson–gluon fusion (figure 6)[20],

$$\gamma g \to \bar{t}t, \; W^- g \to \bar{t}b. \tag{32}$$

The best way to look for top is again through its leptonic decay channel (eq. 29) using isolation. Since the leptonic branching ratio is about 10%, one needs at least 50–100 top quark events for its identification. This corresponds to a top quark discovery limit of about 60 GeV at the HERA collider, for the expected centre of mass energy of 300 GeV and luminosity of 200 events/picobarn.

SUMMARY

The main results are summarized in figure 7.

(i) There are indirect evidence for t quark from the observed forward–backward asymmetry in $e^+e^- \to \bar{b}b$ as well as the absence of flavour changing neutral current decay of b quark.

(ii) There are indirect constraints on t quark mass from (1) the radiative correction to W, Z boson masses, and (2) the $B_d - \bar{B}_d$ mixing. They imply $40\ \text{GeV} < m_t < 200\ \text{GeV}$.

(iii) The e^+e^- colliders are the cleanest machines for direct t quark search; but unfortunately their energies are rather low. The results from PETRA and TRISTAN imply $m_t > 26$ GeV. The LEP (I) and LEP (II) can extend the probe to 50 and 100 GeV respectively.

(iv) The $\bar{p}p$ colliders are more promising because of their higher energy reach. Although the t signal is dirty, it can be cleaned up considerably using the isolated electron (or muon) signature. The result from the CERN $\bar{p}p$ collider suggests $m_t > 40$ GeV. The ongoing searches at the Tevatron and the upgraded CERN colliders are expected to probe t mass up to nearly 100 GeV. The search can be extended to 200 GeV at the upgraded Tevatron collider, scheduled for the mid-ninties.

(v) Thus one expects to see the t quark within a year if its mass is within 100 GeV. If not one has to wait for at least five years.

ACKNOWLEDGEMENT

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5 June 1989

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STRUCTURE OF *bis* (2,2′-BIPYRIDYL) *bis* (2,4,6-TRINITROPHENOLATO)-CALCIUM(II) [$Ca(C_{10}N_2H_8)_2$ $(C_6N_3O_7H_2)_2$]

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ABSTRACT

[Ca $(C_{10}N_2H_8)_2$ $(C_6N_3O_7H_2)_2$], $M_r = 808.7$, m.p. = 488–498 K, orthorhombic, *Pbca*, $a = 24.154(5)$, $b = 16.128(5)$, $c = 18.068(6)$ Å, $v = 7038.5$ Å^3, $Z = 8$, $D_c = 1.528$, $D_m = 1.532$ g cm (flotation), λ (Mo K_α) = 0.7107 Å, Zr filter, $\mu = 2.53$ cm^{-1}, $F(000) = 3312$, room temperature, $R = 0.068$ for 1263 observed reflections. Calcium is eight-coordinated to the four N atoms of 2,2′-bipyridines (bipy) and to the four O atoms belonging to the two trinitrophenolates (tnp) in a distorted square antiprism geometry. In the coordination polyhedra, distances between Ca and phenolic O atoms are shorter than the other Ca–O and Ca–N bond distances. The nitro groups are rotated out of the ring planes of tnp. In both bipy molecules the rings are in the twisted conformation.

INTRODUCTION

IN continuation of our studies on the coordination properties of alkali and alkaline earth cations with respect to various ligands (particularly with the coordinating capacity of nitrophenols[1-3]), crystal structure determination of the title compound was undertaken.

EXPERIMENTAL

Crystals prepared by mixing calcium trinitrophenolate and 2,2′-bipyridine in 1:1 ratio in a solvent with equal amounts of acetone and ethanol; slow evaporation at room temperature yields yellow crystals. Intensity data (crystal size 0.56 × 0.25 × 0.73 mm^3) collected using indigenously fabricated computer-controlled four-circle diffractometer, Zr-filtered Mo K_α radiation, ω-2θ step scan, 3889 reflections recorded in the range $3 \leqslant 2\theta \leqslant 42°$, 1263 with $I > 2.5\,\sigma(I)$ considered observed, index range h 0→22, k 0→16, l 0→16. Corrections applied for Lp and absorption, maximum and minimum values of absorption 0.939 and 0.834 respectively. Cell parameters from least-squares refinement of setting angles of 25 reflections (θ range 8–15°), two check reflections for every 100 data reflections did not vary significantly over the course of data collection, Patterson and Fourier methods, all hydrogens located from difference maps, structure refinement by least-squares based on F values with anisotropic thermal parameters for non-hydrogen atoms and isotropic for hydrogen atoms. $R = 0.067$, $Rw = 0.097$ with $w = 0.5641/[\sigma^2(F) + 0.01058\,F^2]$; $\Delta/\sigma(\max) = 0.63$; $\Delta\rho$ in final difference map -0.3 to $0.4\,e$ Å^{-3}, C–H distances in the range 0.916(16) to 1.224(14) Å, e.s.d. are found to be large, may be due to poor ratio of observation to the number of variables (non-hydrogen 513 variables; hydrogens 80 variables); programs SHELX-76[4], PLUTO[5]; computer NORSK DATA; atomic scattering factors those from SHELX-76.

DISCUSSION

The final positional parameters are given in table 1. The molecular structure of the compound is shown in figure 1. The Ca atom is eight-coordinated by four N atoms of the two bidentate bipy ligands, two O atoms of the phenolic and two O atoms of the *o*-nitro group of the two tnp moieties (figure 2). The symmetry of the coordination polyhedra was examined by the Lippard and Russ[6] test. The angle between intersecting trapezoidal best planes for the polyhedra was 85.4°(9). On these grounds the coordination is an intermediate case between the ideal square antiprism (77.4°) and the ideal dodecahedron (90°). The additional test based on the comparison of average distance of the ligand atoms to

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†For correspondence

calcium coordination; bipyridyl; phenolate; organometallic

Table 1 *Final atomic coordinates (× 10^4) and equivalent temperature factors ($Å^2 \times 10^3$) with e.s.d.*

	x	y	z	U_{eq}
Ca (1)	1781 (1)	665 (2)	2633 (2)	42 (3)
(tnp1)				
O (2)	1438 (4)	51 (6)	1585 (5)	54 (6)
C (3)	1025 (7)	−360 (9)	1362 (9)	51 (8)
C (4)	923 (6)	−484 (8)	580 (8)	42 (7)
N (5)	1356 (5)	−270 (9)	14 (8)	56 (7)
O (6)	1826 (5)	−408 (8)	209 (6)	93 (7)
O (7)	1179 (5)	15 (8)	−567 (7)	85 (7)
C (8)	440 (7)	−874 (9)	271 (8)	53 (7)
C (9)	42 (6)	−1161 (9)	801 (9)	48 (7)
N (10)	−467 (6)	−1590 (9)	521 (9)	71 (8)
O (11)	−523 (5)	−1626 (9)	−149 (8)	110 (7)
O (12)	−769 (5)	−1887 (7)	977 (7)	75 (7)
C (13)	85 (6)	−1049 (9)	1561 (9)	52 (8)
C (14)	573 (6)	−666 (11)	1799 (8)	56 (8)
N (15)	602 (5)	−548 (9)	2627 (8)	66 (7)
O (16)	1063 (4)	−469 (7)	2921 (6)	63 (6)
O (17)	173 (5)	−556 (8)	2963 (6)	89 (7)
(tnp2)				
O (2′)	898 (3)	1278 (7)	2646 (5)	53 (5)
C (3′)	487 (6)	1398 (10)	3070 (9)	51 (6)
C (4′)	478 (6)	1285 (9)	3878 (9)	47 (7)
N (5′)	984 (6)	965 (8)	4224 (8)	61 (7)
O (6′)	1450 (4)	1013 (7)	3907 (6)	65 (6)
O (7′)	942 (6)	686 (9)	4857 (7)	106 (7)
C (8′)	−40 (6)	1432 (9)	4344 (7)	45 (7)
C (9′)	−447 (6)	1713 (8)	4019 (9)	52 (7)
N (10′)	−892 (6)	1938 (10)	4496 (9)	80 (9)
O (11′)	−1305 (5)	2308 (8)	4336 (7)	96 (7)
O (12′)	−900 (6)	1748 (9)	5127 (8)	121 (8)
C (13′)	−487 (7)	1876 (10)	3301 (10)	65 (8)
C (14′)	−62 (8)	1673 (10)	2845 (10)	70 (8)
N (15′)	−84 (7)	1841 (12)	2025 (11)	89 (8)
O (16′)	−193 (7)	1229 (11)	1668 (8)	141 (10)
O (17′)	−29 (8)	2475 (11)	1782 (9)	179 (10)
(bipy1)				
N (18)	2661 (5)	80 (8)	2057 (6)	42 (6)
C (19)	2829 (7)	382 (10)	1409 (8)	53 (7)
C (20)	3401 (8)	302 (10)	1153 (9)	69 (8)
C (21)	3761 (7)	−169 (12)	1627 (10)	72 (9)
C (22)	3562 (7)	−463 (11)	2285 (9)	70 (9)
C (23)	3021 (6)	−364 (10)	2513 (10)	55 (8)
C (24)	2800 (5)	−732 (9)	3178 (7)	35 (7)
N (25)	2313 (5)	−388 (7)	3439 (7)	54 (8)
C (26)	2106 (7)	−701 (10)	4071 (8)	59 (9)
C (27)	2359 (7)	−1356 (11)	4455 (8)	65 (9)
C (28)	2833 (7)	−1699 (10)	4145 (9)	68 (9)
C (29)	3076 (7)	−1392 (11)	3529 (8)	65 (9)
(bipy2)				
N (18′)	2568 (5)	1637 (7)	2995 (6)	47 (8)
C (19′)	2921 (6)	1485 (10)	3594 (9)	62 (7)
C (20′)	3364 (6)	1977 (11)	3759 (8)	50 (6)
C (21′)	3526 (6)	2598 (12)	3319 (9)	60 (7)
C (22′)	3187 (6)	2715 (9)	2702 (7)	44 (6)
C (23′)	2724 (7)	2277 (9)	2530 (9)	57 (6)
C (24′)	2367 (6)	2413 (10)	1862 (8)	42 (6)
N (25′)	1924 (5)	1909 (8)	1838 (6)	51 (7)
C (26′)	1558 (7)	2072 (9)	1258 (9)	60 (7)
C (27′)	1623 (7)	2665 (11)	789 (9)	63 (7)
C (28′)	2083 (7)	3201 (10)	806 (8)	63 (7)
C (29′)	2472 (7)	3025 (9)	1383 (8)	54 (6)

$U_{eq} = (U_{11} + U_{22} + U_{33})/3$.

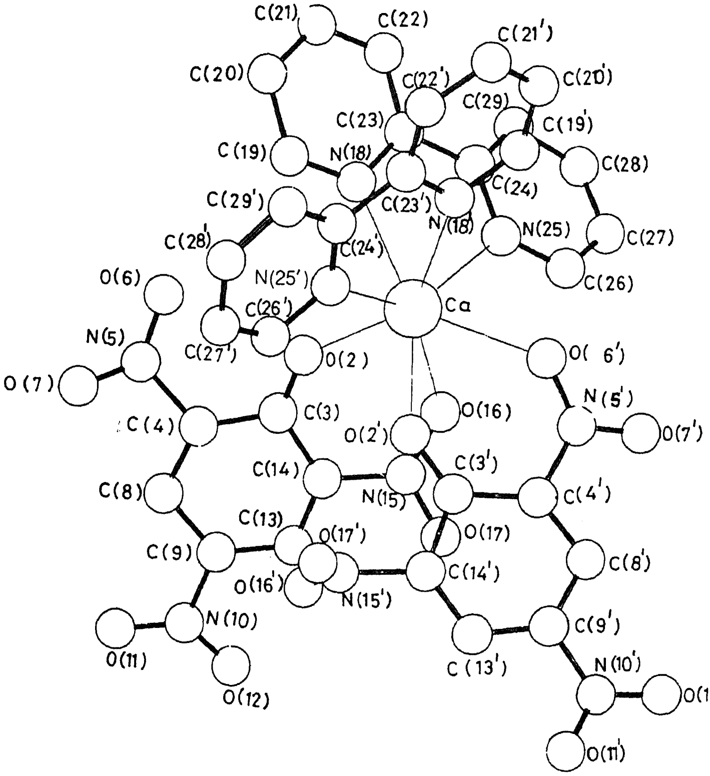

Figure 1. Molecular structure of *bis*(2,2′-bipyridyl)*bis*(2,4,6-trinitrophenolato)calcium(II).

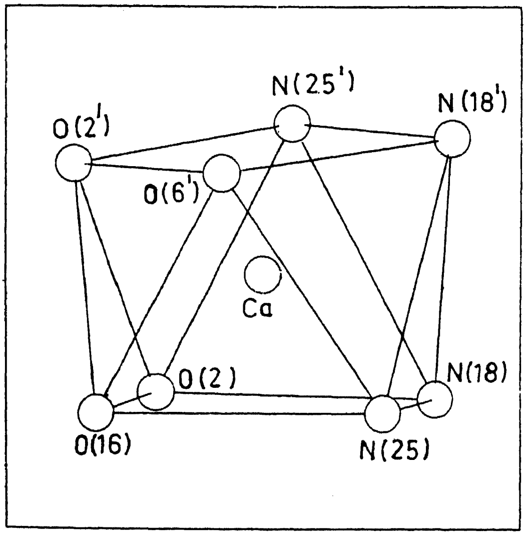

Figure 2. Calcium coordination in *bis*(2,2′-bipyridyl)-*bis*(2,4,6-trinitrophenolato)calcium(II).

trapezoidal planes [$\mathrm{d}T = 0.275(15)$, $\mathrm{d}T = 0.314(14)$ Å] with average distance of the ligand atoms to the best planes through the square faces of the antiprism [$\mathrm{d}S = 0.19(8)$, $\mathrm{d}S = 0.023(9)$ Å] allows us to describe the polyhedra as distorted square antiprisms. Table 2 gives bond lengths and bond angles. The distances between Ca and phenolic O(2) and O(18) atoms [2.292(10), 2.351(9) Å] are smaller than the other Ca–O and Ca–N distances, which lie in the range 2.492(12)–2.578(12) Å. Calcium displays two properties, viz. anionphilicity by virtue of which it interacts strongly with the phenoxide and a strong polarizing ability towards the neutral N atoms of bipy compared to O atoms of NO group of tnp. The O–Ca–O bite angles of tnp [66.8(3), 67.5°(3)] are observed to be greater than the bite angles N–Ca–N of bipy [64.6(4) and 63.2°(4)]. The bond lengths and angles of the benzene ring of the two tnp's agree with those reported in many nitrophenolate complexes[7–9] and show common features such as larger C–C bond lengths and smaller C–C–C angles at the

Table 2 *Bond lengths (Å) and angles (degrees) with e.s.d.*

Ca (1)–O (2)	2.292 (10)	O (2)–O (16)	2.712 (12)
Ca (1)–O (2′)	2.351 (9)	O (16)–N (25)	3.164 (13)
Ca (1)–O (16)	2.574 (11)	N (25)–N (18)	2.741 (13)
Ca (1)–O (6′)	2.502 (10)	N (18)–O (2)	3.075 (14)
Ca (1)–N (18)	2.547 (11)	O (2′)–O (6′)	2.675 (14)
Ca (1)–N (25)	2.579 (12)	O (6′)–N (18′)	3.321 (14)
Ca (1)–N (18′)	2.550 (12)	N (18′)–N (25′)	2.643 (13)
Ca (1)–N (25′)	2.492 (12)	N (25′)–O (2′)	3.050 (13)
	(tnp1)	(tnp2)	
O (2)–C (3)	1.265 (18)	1.270 (17)	
C (3)–C (4)	1.448 (21)	1.472 (23)	
C (4)–C (8)	1.439 (21)	1.372 (20)	
C (8)–C (9)	1.433 (20)	1.392 (21)	
C (9)–C (13)	1.388 (21)	1.327 (24)	
C (13)–C (14)	1.398 (20)	1.356 (25)	
C (14)–C (3)	1.435 (21)	1.457 (23)	
C (4)–N (5)	1.502 (19)	1.467 (20)	
C (9)–C (10)	1.499 (20)	1.425 (22)	
C (14)–N (15)	1.511 (20)	1.507 (26)	
N (5)–O (6)	1.211 (17)	1.266 (17)	
N (5)–O (7)	1.223 (18)	1.233 (18)	
N (10)–O (11)	1.218 (20)	1.258 (19)	
N (10)–O (12)	1.201 (20)	1.181 (20)	
N (15)–O (16)	1.239 (16)	1.209 (25)	
N (15)–O (17)	1.202 (18)	1.121 (27)	
	(bipy1)	(bipy2)	
N (18)–C (19)	1.332 (18)	1.400 (19)	
C (19)–C (20)	1.463 (23)	1.365 (21)	
C (20)–C (21)	1.437 (24)	1.337 (23)	
C (21)–C (22)	1.369 (24)	1.397 (21)	
C (22)–C (23)	1.378 (22)	1.359 (21)	
C (23)–N (18)	1.395 (19)	1.383 (19)	
C (23)–C (24)	1.444 (21)	1.499 (21)	
C (24)–N (25)	1.382 (18)	1.344 (18)	
N (25)–C (26)	1.345 (19)	1.395 (20)	
C (26)–C (27)	1.403 (23)	1.287 (22)	
C (27)–C (28)	1.390 (23)	1.408 (23)	
C (28)–C (29)	1.352 (22)	1.433 (22)	
C (29)–C (24)	1.408 (21)	1.336 (20)	
O (2)–Ca (1)–O (16)	67.5 (3)	O (2)–O (16)–N (25)	86.2 (6)
N (18)–Ca (1)–N (25)	64.6 (4)	O (16)–N (25)–N (18)	92.1 (8)
O (2′)–Ca (1)–O(6′)	66.8 (3)	N (25)–N (18)–O (2)	87.3 (8)
N (18′)–Ca (1)–N (25′)	63.2 (4)	N (18)–O (2)–O (16)	94.5 (6)
O (2)–Ca (1)–O (2′)	82.1 (3)	O (2′)–O (6′)–N (18′)	86.2 (6)
O(2′)–Ca(1)–O(16)	71.7(3)	O (6′)–N (18′)–N (25′)	87.9 (8)
O (16)–Ca (1)–O (6′)	76.0(3)	N (18′)–N (25′)–O (2′)	92.6 (6)
O (6′)–Ca (1)–N (25)	77.7(4)	N(25′)–O(2′)–O(6′)	93.0 (6)
N (25)–Ca (1)–N (18′)	83.6 (4)		
N (18′)–Ca (1)–N (18)	73.2 (4)		
N (18)–Ca (1)–N (25′)	87.0 (4)		
N (25′)–Ca (1)–O (2)	85.5 (4)		
	(tnp1)	(tnp2)	
O (2)–C (3)–C (4)	111.1 (13)	126.3 (13)	
C (3)–C (4)–C (5)	121.0 (13)	117.0 (13)	
C (4)–N (5)–O (6)	115.3 (12)	121.8 (12)	
C (4)–N (5)–O (7)	114.3 (13)	117.2 (13)	
O (6)–N (5)–O (7)	130.3 (14)	120.9 (14)	
N (5)–C (4)–C (8)	113.6 (12)	116.2 (14)	
C (3)–C (4)–C (8)	125.2 (13)	126.8 (13)	
C (4)–C (8)–C (9)	115.3 (12)	116.6 (13)	
C (8)–C (9)–N (10)	118.2 (13)	117.7 (14)	

Table 2. (*Contd.*)

C (9)–N (10)–O (11)	116.6 (14)	118.8 (15)
C (9)–N (10)–O (12)	116.8 (14)	122.1 (15)
O (11)–N (10)–O (12)	126.5 (15)	118.8 (15)
N (10)–C (9)–C (13)	117.1 (13)	119.1 (14)
C (8)–C (9)–C (13)	124.6 (13)	122.6 (15)
C (9)–C (13)–C (14)	115.1 (12)	119.3 (16)
C (13)–C (14)–N (15)	113.6 (12)	121.8 (15)
C (14)–N (15)–O (16)	118.7 (12)	112.6 (16)
C (14)–N (15)–O (17)	117.2 (12)	123.1 (18)
O (16)–N (15)–O (17)	124.0 (14)	124.2 (20)
N (15)–C (14)–C (3)	117.7 (13)	111.1 (14)
C (13)–C (14)–C (3)	128.6 (14)	126.5 (16)
C(14)–C(3)–C(4)	127.3 (14)	107.5 (13)
C (14)–C (3)–O (2)	127.3 (14)	126.2 (14)
	(bipy1)	(bipy2)
N (18)–C (19)–C (20)	122.2 (13)	105.8 (12)
C (19)–C (20)–C (21)	115.4 (14)	122.4 (14)
C (20)–C (21)–C (22)	119.3 (15)	113.8 (14)
C (21)–C (22)–C (23)	123.4 (16)	126.6 (14)
C (22)–C (23)–N (18)	118.3 (14)	118.2 (14)
C (23)–N (18)–C (19)	121.2 (12)	115.8 (12)
C (22)–C (23)–C (24)	123.5 (14)	125.6 (14)
N (18)–C (23)–C (24)	118.1 (12)	116.3 (13)
C (23)–C (24)–N (25)	123.6 (12)	113.3 (12)
C (24)–N (25)–C (26)	117.1 (12)	114.5 (12)
N (25)–C (26)–C (27)	126.6 (14)	123.8 (15)
C (26)–C (27)–C (28)	117.4 (14)	122.6 (15)
C (27)–C (28)–C (29)	122.9 (15)	114.3 (14)
C (28)–C (29)–C (24)	116.2 (14)	119.5 (15)
C (29)–C (24)–N (25)	123.6 (12)	125.2 (13)
C (29)–C (24)–C (23)	120.7 (12)	121.3 (13)

phenolic sites, and enlarged angles (126°) at *ortho* C atoms. The phenyl rings are moderately planar, the σ_{plane}, defined as $[\sum_i d_i^2/(n-3)^{1/2}]$, where d is the distance of atom from plane and n is the number of atoms in the plane, being 0.006(6) and 0.026(8) Å. The nitrogens (ring substitutions) show more deviations from the least-squares plane in tnp1 [N(5), 0.12(1); N(10), 0.06; N(15), −0.04(1) Å] than in tnp2 [N(5′), 0.01(1); N(10′), 0.10(1); N(15′), 0.00(1) Å]. Apart from bending relative to the plane of the benzene ring, the planes of the nitro group display various degrees of twisting—moderate for *para* nitro groups and large for *ortho* nitro groups [in tnp1 7.2(7), 39.4(8), 23.6°(8); in tnp2 10.7(1), 18.5(8), 83.8°(8)], 83.8° being the largest twist reported so far. The two tnp planes are inclined at 55.9°(8) to each other. Tables of mean planes, torsional angles and F_o, F_c are available from the authors. In the two bipys, the intraligand twist angle (based on parameters defined by Cordes *et al.*[10]) were found to be 19.4(7) and 5.4°(7), and χ_c values were found to be 3.4 and 1.4°. This suggests that in both the bipys the rings are in the twisted conformation and not in the bowed conformation. The packing of the molecules shows no abnormally short contacts.

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ANNOUNCEMENTS

New Drug Strategies in the Prevention and Treatment of Stroke

Date: 24–25 January 1990
Place: London

Stroke occurs commonly and with devastating consequences. Patients are often left dependent on expensive nursing care, and in need of years of rehabilitation therapy. Is there not more that can be done to prevent stroke or to minimise the damage it wreaks?

This international conference will examine drug strategies that have been tried in the past, and novel pharmacological approaches currently under study, in both the prevention and the treatment of acute stroke. Experts in the field will discuss the latest developments, reviewing results from large clinical studies and examining the progress that is being made on the experimental front. The meeting, aimed specifically at all those involved in testing and developing new drugs for use in stroke, will provide an invaluable overview of present day management of the condition, and changes that the future may bring.

Osteoporosis—Prospects for Prevention and Treatment

Date: 5–6 October 1990
Place: London

The objective of this meeting is to bring together leading authorities, to discuss basic research and clinical studies related to osteoporosis. Oestrogens play a central role in preventing bone loss. The role of hormone replacement therapy in postmenopausal women and its associated risks and benefits will be discussed. The strategies and techniques available for screening and designing trials will also be discussed. For the future, there is a strong need to develop new pharmacological agents that will stimulate new bone formation and enhance bone mass. The new leads in this direction will be reviewed, as well as experimental approaches for their evaluation.

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SOLVENT EFFECTS ON THE ELECTRIC MOMENTS OF SOME HEXAHYDRO-s-TRIAZINES

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ABSTRACT

Molar polarizations ana permanent electric moments of unsubstituted 1,3,5-triphenyl hexahydro-s-triazine (HsT) and the -methyl, -chloro, -methoxy, -ethoxy and -nitro, tri *p*-phenyl substituted derivatives of HsT have been determined in nine different solvents with permittivity range $\varepsilon_s = 1.8$ to 32.0. A positive solvent effect has been detected and the polarizability ellipsoid of the molecules envisaged. The correlation parameter (g) values seem to linearly depend on solvent ε_s and the Hammett substituent (σp) constant values of HsT derivatives.

INTRODUCTION

The permanent electric moments (μ) and molar polarizations of unsubstituted and *p*-phenyl substituted derivatives of 1,3,5-triphenyl hexahydro-*s*-triazines (HsT) were reported earlier[1]. It is well known that values of P and μ evaluated by the dilute solution method are generally solvent-dependant and differ from gas phase values[2,3]. For solutes like HsT and its derivatives whose decomposition inception lies between m.p and b.p range, the gas phase (μ_g) determinations prove difficult. The same is overcome by the extrapolation of ε_s vs μ_s plots assuming $\mu_s \rightarrow \mu_g$ as $\varepsilon_s \rightarrow 0$. The ratio μ_s/μ_g gives the correlation parameter g which is a measure of molecular interactions[4,5]. Data based on these lines are not available in the literature for molecules like HsT which possess certain stereochemical characteristics. Hence, a rationalization on the static dielectric data of HsT compounds in various solvents, has been presented here. Compounds of the study are: 1,3,5[-triphenyl; -tri(-4-methyl phenyl); -tri(-4-chlorophenyl); -tri(-4-methoxy phenyl); -tri(4-ethoxy phenyl) and -tri(4-nitro phenyl)] hexahydro-*s*-triazines and the same are denoted as I, II, III, IV, V and VI respectively.

EXPERIMENTAL

Solutes I–VI were prepared and recrystallized as mentioned elsewhere[6]. The solvents employed belong to spectro grade E. Merck samples. Static permittivity values were computed on a PICO-dipolmeter at 7 MHz frequency. A 10 ml capacity pyknometer and an Abbe refractometer were used for density and refractivity studies. In all measurements 25°C was kept constant by an ultrathermostat fitted with external circulation.

RESULTS AND DISCUSSION

Correlation parameters

Evaluation of g of the solutes I–VI needs the solute μ_g values. Hence the dipole moment of the solute in the solution (μ_s) from various solvents was determined, employing Guggenheim's dilute solution method with the solute concentration (x_2) not exceeding 0.09. Using the $\varepsilon_{1,2}$, $d_{1,2}$ and P_1 data, the total molar polarization of the solutes ($P_{2\infty}$) was determined from the plots of P_2 vs x_2 as $x_2 \rightarrow 0$. Similarly the electronic polarization P_e was determined from the R_2 vs x_2 plot, as $x_2 \rightarrow 0$, $R_2 \rightarrow R_{2\infty}$ where $R_2(\infty)$ is the total molar refraction of the solute. The induced polarization $P_i = P_e + P_a$ where $P_e = R_{2(\infty)}$ and $P_a \simeq 0.15\, P_e$ respectively. The orientation polarization was $P_0 = P_{2\infty} - P_i$. From the μ_s values determined from the P_0 values in each of the solvent, μ_s vs ε_s plots were made wherein $\mu_s \rightarrow \mu_g$ as $\varepsilon_s \rightarrow 0$. The ratio μ_s/μ_g gives the g value. Excluding non-polar solvents like C_6H_6 and CCl_4, the g value for the polarizable dipole placed in a continuous polar medium of permittivity ε_s is given by Onsager's model as,

$$g = \frac{3\varepsilon_s(n_2^2+2)}{(n_2^2+2\varepsilon_s)(\varepsilon_s+2)}. \quad (1)$$

However, the g values from equation (1) and the

Table 1 *Correlation (g) and form factor (F) values of HsTs at 25°C*

	I			II			III			IV			V			VI		
Solvent	g	g_0	F	g	g_0	F	g	g_0	F	g	g_0	F	g	g_0	F	g	g_0	F
n-hexane	1.05	1.07	0.706	1.03	1.04	0.611	0.95	0.95	0.561	1.03	1.04	0.555	1.01	1.02	0.531	1.01	1.02	0.527
CCl_4	1.06	1.06	0.707	1.04	1.06	0.600	0.96	0.97	0.565	1.04	1.05	0.541	1.02	1.04	0.530	1.02	1.03	0.525
C_6H_6	1.07	1.08	0.705	1.08	1.07	0.615	0.97	0.98	0.562	1.05	1.04	0.545	1.04	1.05	0.535	1.03	1.04	0.528
$CHCl_3$	1.17	1.15	0.708	1.17	1.15	0.622	0.99	0.98	0.610	1.11	1.10	0.557	1.08	1.07	0.541	1.05	1.06	0.531
n-BuCl	1.23	1.24	0.810	1.26	1.25	0.633	1.03	1.05	0.625	1.17	1.18	0.562	1.13	1.12	0.546	1.08	1.07	0.538
n-BuOH	1.64	1.64	0.815	1.68	1.67	0.637	1.20	1.21	0.636	1.50	1.51	0.568	1.33	1.35	0.551	1.22	1.21	0.541
Acetone	1.73	1.75	0.822	1.80	1.79	0.642	1.24	1.25	0.638	1.60	1.62	0.620	1.39	1.38	0.553	1.24	1.23	0.546
EtOH	1.87	1.86	0.830	1.94	1.95	0.651	1.30	1.31	0.642	1.70	1.71	0.633	1.45	1.46	0.558	1.30	1.29	0.550
MeOH	2.01	2.00	0.841	2.02	2.01	0.662	1.35	1.35	0.650	1.80	1.79	0.645	1.50	1.49	0.564	1.33	1.32	0.557

g_0 values refer those from Onsager's theory.

(μ_s/μ_g) method given in table 1 seem comparable. It is observed that for all compounds I–VI a positive solvent effect is seen as $\mu_s > \mu_g$.

From the existing theories of solvent effect[7,8] a positive solvent effect can be attributed to a molecular dipole directed along the minor axis of an ellipsoidal molecule. In the cse of a HsT molecule (figure 1) the induced moment due to polarization of the *n*-electron cloud by the solvent dipoles should lie along the minor molecular axis, causing an increase in the resultant dipole moment (μ_s) of the molecule compared to μ_g. Apart from this induced polarization contribution, even orientation polarization contribution could be envisaged for HsT type molecule where relative proportions of chair and boat conformers in solution determine the resultant μ_s. Since the boat conformer (figure 1) has a lower μ value (because the lone pairs do not lie on the same direction) if the solvent polarity (ε_s) increases the proportions of chair conformer in the solution, an increase in P_0 and thus an increase in μ_s, could be seen. Assuming the HsT molecules to be ellipsoids, the form factor F values[9,10] which are directly related to the ratio of the semi-major and minor axes of the ellipse could be evaluated using the Ross and Sack relationships

$$g = \frac{\mu_s}{\mu_g} = \frac{3\varepsilon_s[1-(n_2^2-1)F]}{(\varepsilon_s+2)[\varepsilon_s+(n_2^2-\varepsilon_s)F]}, \tag{2}$$

and

$$g-1 = \frac{\Delta\mu}{\mu_g} = \frac{(\varepsilon_s-1)[(\varepsilon_s+2n_2^2)F-\varepsilon_s]}{(\varepsilon_s+2)[\varepsilon_s+(n_2^2-\varepsilon_s)F]}. \tag{3}$$

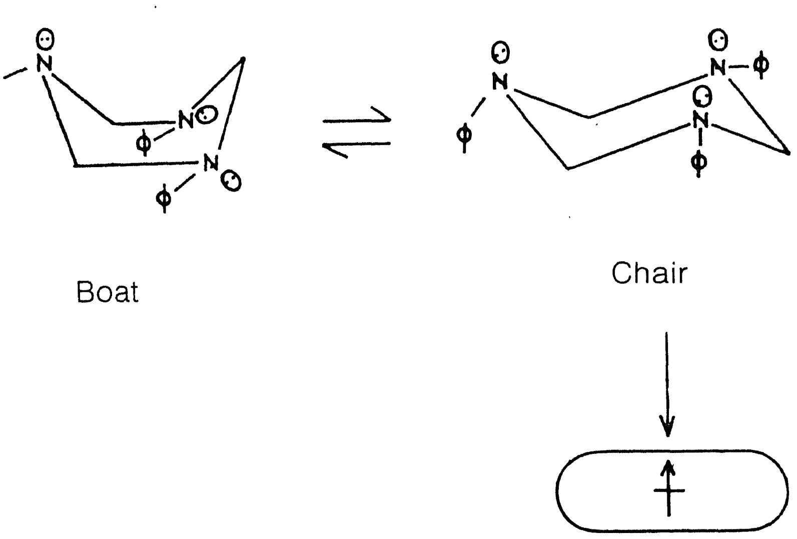

Figure 1. Molecular orientations of HsT.

Since equations (2) and (3) have been derived based on the Onsager's model, F values of the solutes I–VI were determined using the g values obtained from (1). The F values (table 1) seem to lie in the range reported for small ellipsoidal molecules.

Linear dependances

Many authors have attempted to express solvent effects in terms of anisotropy of the solute molecular polarizability[11, 12]. The experimental evidence supports an equation of the form $(g-1)=A(\varepsilon_s-1)/C$ where C is either a constant or slightly dependent on ε_s and A depends on the shape of the molecule and the position of the dipole within it. For a dipole placed symmetrically at the centre of the molecule with the major axis perpendicular to the dipole axis, the slope (A/C) has a positive value, confirming a positive solvent effect. Since $g=\mu_s/\mu_g$, and the resultant dipole moment of the HsT molecule depends on the +I and −I effect of the *para*-phenyl substituents, g should exhibit a linear dependence on the Hammett substituent (σ_p) values. Figures 2a and 2b exhibit both the g effects.

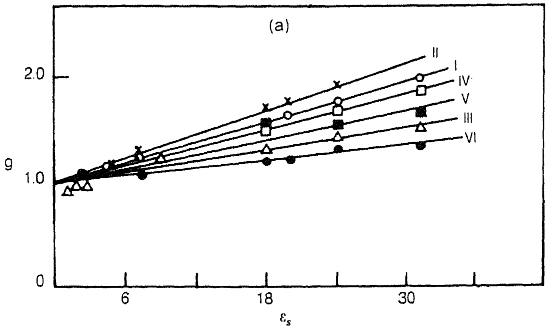

Figure 2a. g vs ε_s plots I–VI HsTs.

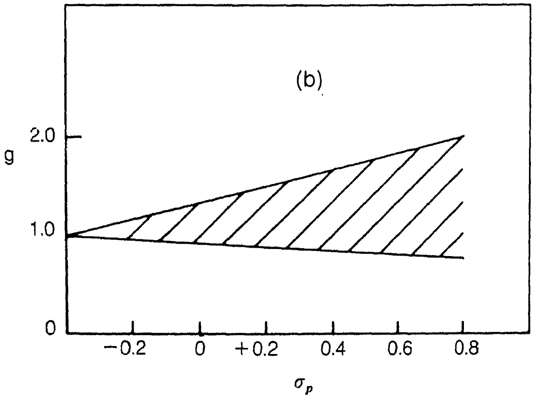

Figure 2b. Region g variation on σ_p for I–VI in 9 solvents.

ACKNOWLEDGEMENT

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ROOT-MEDIATED WATER TRANSPORT TO THE SHOOT IN RICE

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ABSTRACT

Rice leaf rolled quickly and its water potential and relative water content decreased immediately following excision of a well-watered plant even though the excised plant stood in water. Externally applied pressure to force entry of water into the excised plant resulted in unrolling of leaf with concomitant elevation of leaf water status as did the supply of water to the root system of a previously stressed intact plant. Thus, in intact rice plant, roots appear to possess the capacity of pushing water up in the shoot. However, in the absence of seminal or basal root system, the function of bulk water uptake and transport is taken over by nodal roots. It is suggested that the bulk of the water in rice plants is essentially propelled upward by roots alone, not by water potential gradient between the top and bottom of the plant. To accomplish this the intact rice plant seems to generate pressure in the root.

INTRODUCTION

THERE is a continuum of liquid water from the soil through root and stem to the leaves, and a continuum of water vapour from the intercellular spaces in the leaves, across leaf epidermis, and through the air boundary layer to the atmosphere[1,2]. The liquid continuum, which is maintained by continuous absorption and transport of water, constitutes the transpiration stream[3]. It is generally accepted that the absorption and transport of water are passive movement, downhill in terms of the free energy status of the water or water potential[4,5]. It then follows that leaf and shoot water potentials must be lower than root and soil water potentials for absorption and transport of water to the shoot, which in fact do occur, at least during the daytime, because transpiration often exceeds absorption[6]. Most workers agree that transpiration, by reducing leaf water content and water potential, gives rise to the water potential gradient for uptake[7]. Thus water uptake is considered as the consequence of water loss[8].

In this communication we present evidence which demonstrates that the bulk of water in rice is propelled upward in the shoot by some mechanism located in the roots and not by water potential gradient existing between the top and bottom of the plant.

MATERIALS AND METHODS

Rice plants (cv. Sarjoo-52) were grown in earthen pots of 15 kg capacity with adequate fertilizers and water under open-air greenhouse conditions. When the plants were 3 months old some of them were subjected to water stress by withholding supply of water until leaf water potential fell to −2.7 MPa. At this stage some pots were shifted to the laboratory and similar-looking tillers from well-watered pots were excised at the base under water, with panicle and only the top two leaves intact. Approximately 8 cm of the base of the excised tiller was gently inserted into the neck of a laboratory pressure stove through a rubber cork and the arrangement was made fully air-tight (see figure 1). These operations were carried out under water contained in a large tub. The tank of the stove was filled with water and fitted with a pressure gauge to monitor the pressure applied to force entry of water into the excised tiller through the cut end. Concurrently stress on plants previously subjected to water stress was released by supplying water to the root medium. Immediately after supplying water some pre-stressed tillers were also excised at the base under water in the pot and held in that position to allow recovery from the water stress. Observations of second leaf unrolling[9], and measurements of water potential[6] and relative water content[10] were made between short intervals after applying a pressure of +0.25 MPa in the case of excised watered plants and from the time of stress relief in intact stressed as well as excised stressed plants. By the time pressure was applied the leaf water potential had declined to −2.7 MPa.

In another set of experiments a few healthy and uniform tillers from well-watered pots were excised and the panicle and all the leaves except the top two

water potential gradient; Nodal roots; Aerenchymato
[illegible]

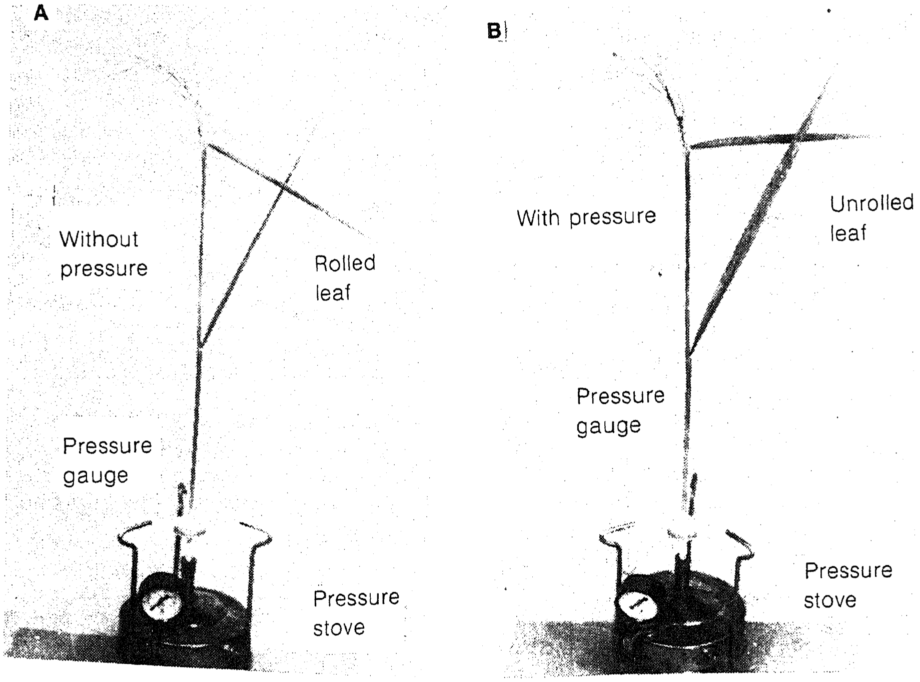

Figure 1. Effect of applied pressure on water transport in excised shoot of rice as evidenced by (A) leaf rolling before pressure application, and (B) leaf unrolling after pressure application.

were removed. The excised tillers were then incubated, keeping them upright under low evaporative demand conditions in the laboratory (ambient temperature 24°C, RH 96% vapour pressure 19.9 mm, wind velocity zero–no air movement), with about 10 cm of the basal portion submerged in water contained in a beaker such that a node remained about 5 cm deep in water. The water was changed every alternate day. Profuse roots, measuring about 10 cm in length, developed at the node submerged under water by day 18. The nodally rooted excised tillers were then taken out and subjected to three types of treatment. In one case all the roots were cut at the point of their origin at the node and the basal cut ends of the rootless tillers were immediately placed in test tubes (15 × 2 cm), which were then filled with water up to the node such that the point of origin of the roots also remained submerged under water. In the second case the roots were left intact but kept out of water by pressing them against the inner wall of the test tube using a thin glass rod. In the third case, the roots were left intact and were fully submerged in water; this served as control. In every case the cut end of the stem remained under water during experimentation. Every test tube was thickly lined with moist white cloth to prevent the nodal roots from overheating in the sun. The entire assembly was then transferred outside the laboratory to the open-air high evaporative demand conditions with ambient temperature 32°C, RH 88%, vapour pressure 21.4 mm and wind velocity 3.4 km per hour. Observations of second leaf rolling[9] were recorded between short intervals.

RESULTS AND DISCUSSION

One of the most immediate effects of plant excision was quick leaf rolling (figure 1,A) accompanied by significant fall in leaf water potential and relative water content even though the cut end of the excised plant remained under water in the pressure stove. Excising the stem and keeping the cut end under water should have enhanced water uptake and transport, preventing leaf rolling, on account of the removal of roots, if, according to Kramer[6], the roots were a major site of resistance to water movement. The inability of excised plants,, though standing in water, to absorb and transport water strongly suggests the direct involvement of roots in these processes. However, the application of external pressure of +0.25 MPa to force entry of water into excised plants through the cut end resulted in complete unrolling of leaves (figure 1, B) and rapid increase in leaf water status within 30 min, including a 10-min lag period (figure 2). Not surprisingly, supply of water to previously stressed, intact potted plants also resulted in quick leaf unrolling, i.e. within 35 min including a 20 min lag period. In addition to leaf unrolling, leaf water potential of excised plants also increased sharply from −2.7 MPa to −0.36 MPa after application of external pressure (figure 3). Similarly, leaf water potential of intact potted plants also increased with the application of water to the root medium and rose to −0.5 MPa within 40 min, including a lag period. The pattern of change in pressure-induced increase in leaf relative water content (figure 4) agreed with that of leaf water potential, suggesting that leaf unrolling and increase in leaf water status were not merely due to increase in physical pressure *per se* in the leaf but in fact due to concurrent increase in leaf water content. Clearly, the case of pressure-induced unrolling of leaf and elevation of leaf water status of excised plants is analogous to the results obtained upon direct water application to intact plants. The reason for the relatively longer lag period (20 min) for leaf unrolling and recovery of leaf water status in the case of intact potted plants could be, partly the resistance to water movement offered by the soil and the plant and partly the adjustment of root metabolic activity to the changed circumstances upon water supply^5. Ignoring the lag, intact plants took only 15 min for complete unrolling of leaf whereas excised plants under external positive pressure of 0.25 MPa took about 20 min. One of the reasons for rapid uptake and transport of water in

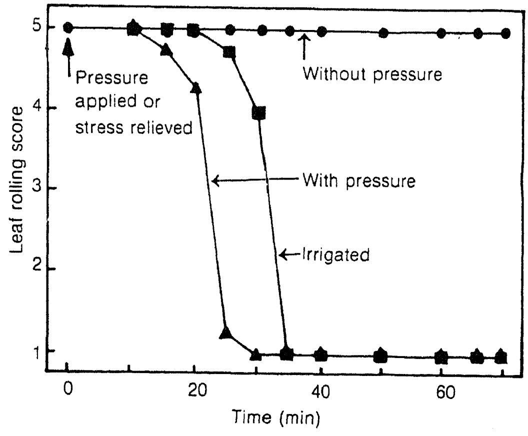

Figure 2. Effect of applied pressure on water transport in excised shoot of rice as evidenced by leaf unrolling. (Score 1, unrolled; score 3, partially rolled; score 5, rolled.)

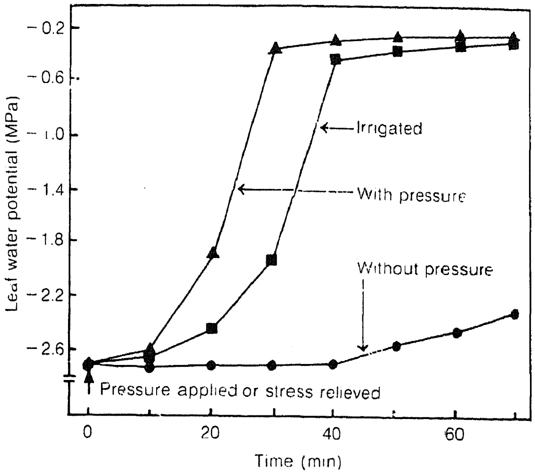

Figure 3. Effect of applied pressure on water transport in excised shoot of rice as evidenced by leaf water potential.

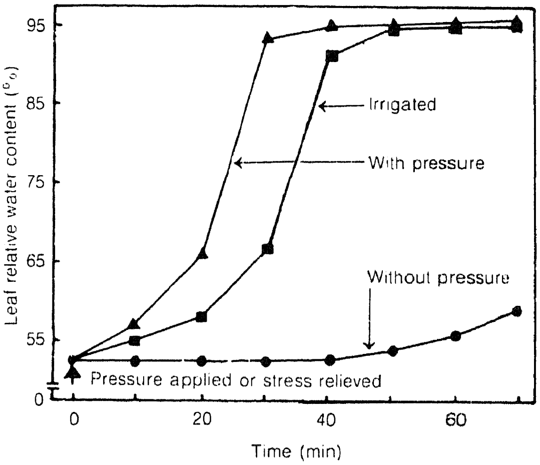

Figure 4. Effect of applied pressure on water transport in excised shoot of rice as evidenced by leaf relative water content.

intact plants could be increased root permeability on account of abscisic acid accumulation[11], generating a pressure in the roots that is greater than the externally applied pressure to excised plants. Generally, root pressure of about +0.1 MPa, and rarely, +0.2 MPa, has been registered in detopped plants, but in intact rice plants it appears to be more than the values reported for other crops[12].

The effects of nodal roots on leaf water relations provide additional evidence in support of roots functioning as a major site of water uptake and generation of force for upward transport of water in rice. Root removal or keeping water out of reach of the roots resulted in almost immediate leaf rolling (only 2–6 min) though the plant stood in water, whereas leaf rolling took 65 min when roots were intact and dipped in water (figure 5). This clearly indicates that roots are essential for water uptake and upward transport of water in the shoot and play an important role in overall water economy of rice plants. Rolling of leaves even in the presence of nodal roots under high evaporative demand conditions simply indicates that the supply of water by the roots was perhaps not enough to cope with loss from the leaves, probably because ascending water has to encounter enormous resistance in the shoot, which has at least 30% aerenchymatous tissues and air cavities[13].

In rice plants, like in other intact transpiring plants, it appears as if water is transported upward on account of water potential gradient between leaves and roots. But the present findings suggest that water reaches the top of rice plants on account of some kind of 'water pump' essentially located in the roots. However, in intact plants it becomes difficult to ascertain the role of roots as a 'pushing force' and therefore the pressure developed by roots *in situ* is not obvious. The anology between pressure-induced transport of water in the excised plant and root-mediated transport in the intact plant strongly suggests the possibility of roots functioning as a pumping machine and not merely as wicks for water supply to the shoot[14,15]. The existence of such a mechanism in rice roots as the sole source of bulk transport of water is further strengthened by the evidence of the inability of rice plants to make osmotic adjustments for leaf turgor maintenance in the event of water deficit[16,17]. Though it is difficult to evaluate the magnitude of such root pressure in intact plants *in situ*, in the light of the present findings it is tempting to estimate that it is at least +0.3 MPa in irrigated rice.

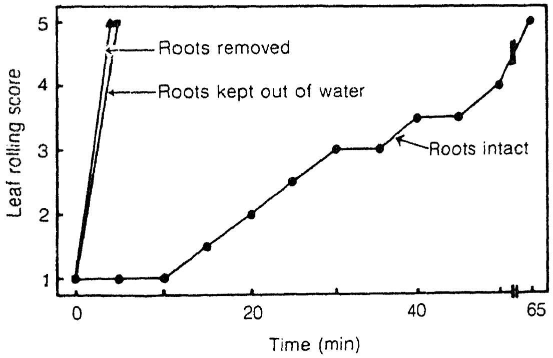

Figure 5. Role of roots in water transport to the shoot as evidenced by leaf rolling in rice.

ACKNOWLEDGEMENT

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11 April 1988

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ANNOUNCEMENT

Airway Hyperreactivity—Is it Really Important for Asthma?

Date: 26–27 February 1990
Place: London

Airway hyperreactivity is a characteristic feature of asthma which is generally thought to be a consequence of chronic airway inflammation. Over the past five years dramatic changes have occurred in the approach adopted by the preclinical groups working in asthma. New models of airway hyperreactivity have been set up in an attempt to analyse the basic underlying mechanism(s) and to identify new therapeutic agents. Asthma therapy is increasingly being directed to the earlier alleviation of airway hyperreactivity by the treatment of the underlying inflammation using prophylactic and anti-inflammatory drugs.

This meeting will review the preclinical and clinical developments in this exciting new field. It will be of benefit to scientists and physicians alike with an interest in this area, as well as those individuals who require an up-to-date overview.

For details contact: Renata Duke, IBC Technical Services Ltd., Bath House (3rd Floor), 56, Holborn Viaduct, UK.

SHORT COMMUNICATIONS

EXCESS ENTHALPY DURING ELECTROLYSIS OF D_2O

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RECENTLY several reports[1-5] discussed the generation of excess enthalpy during the electrolysis of D_2O and suggested the possibility of either nuclear fusion[1,2,6] or chemical reaction[2,7] as the cause of this excess enthalpy. We wish to consider here the enthalpy estimates arising from the adsorption of D_2 ($-\Delta H = 120$ kJ mol^{-1}) and the recombination of D_2 and O_2 in the electrolytic cell ($-\Delta H = 285$ kJ mol^{-1}).

D_2O used in the experiments had a purity of 99.5% (as determined by NMR). The titanium cathode (4″ × 4″) was cleaned and etched to remove oxide films and impurities on the surface of the metal. Platinum sputtered on glass and of the same area as the Ti cathode was used as the anode. Sodium chloride (BDH, Analar grade; 1.16 g) was dissolved in 20 ml of D_2O and used for the electrolysis. The temperature of the electrolytic solution was measured continuously using either a Beckman or a Riege (DDR) thermometer. The cathode surface temperature was measured using a P 85 BIOA. 202 N thermistor (Thermometrics, N. J., USA; $R = 1.845$ kΩ). The electrolytic cell was a 50 ml beaker to which a Perspex plate was fitted to hold the electrodes in parallel configuration. The cell was placed in a Dewar during the experiment. Cooling correction to be applied to the measured temperature was obtained by independently running the electrolysis in the same cell to the steady state temperature and allowing it to cool to the initial temperature. The correction interval was kept the same as the temperature measuring interval, as Newton's law of cooling is valid only for short temperature intervals.

Figure 1 shows the current–voltage curve of D_2O at Ti working electrode. An interesting feature of this curve is the appearance of looping (hysteresis) during the anodic sweep following the cathodic potential sweep to -1.20 V vs SCE. Clearly there is no desorption peak observed during the anodic cycle, as is generally seen. The pronounced hysteresis resulting from adsorption sluggishness has been previously reported[8] for some metals in the electrolysis of H_2O. The features of figure 1 are attributed to specific adsorption of the product of electrolysis[9,10]. Kolotyrkin and co-workers[11-14] and Parsons[15] have examined this behaviour in detail. A particular point to be noted here is that the hysteresis onset is not observed before the cathodic background limit of D_2O is reached; it is likely to be caused by adsorption of D or D_2. The current–voltage curve for H_2O at Ti cathode has also been examined; here hysteresis is not observed (dotted line in figure 1). As predicted by the earlier theories[15,16], the desorption peak of H_2 is seen in the current–voltage curve. The peak shape is broad, indicating a slow desorption of the adsorbed species. This feature is interpreted on the basis of metal–gas physical adsorption based on the Lennard–Jones potential energy diagram[17,18]. Physical adsorption is attributed to long-range forces and it does not involve dissociation of H_2; the heat of adsorption $-\Delta H_p$ is of the same order of

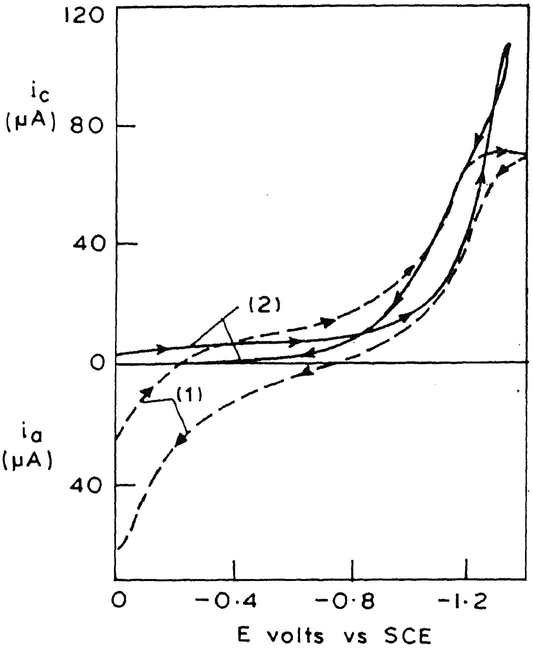

Figure 1. Current–voltage curves for D_2O (solid line) and H_2O (broken line) at Ti working electrode. Sweep rate 0.10 V s^{-1}. Arrows indicate sweep directions.

magnitude as the heat of liquefaction of gaseous H_2 (ref. 18). Chemisorption can also occur and this is discernible in the desorption peak as a shoulder (in the present case it is not observed). The distinction between weak and strong adsorption processes has been investigated previously[18]; these results suggest that strong adsorption occurs only on permitted sites and the sites involve surface atoms. Each surface atom may act as one such site. One half of the available sites may be used for strong adsorption from structural considerations.

Galvanostatic electrolysis of D_2O in a partially closed container containing 1 M NaCl was conducted at 33.0 mA cm^{-2} or 66.0 mA cm^{-2}. The temperature of the electrolytic solution was measured at regular times for a period of 8 h. Figure 2 summarizes the data obtained during the electrolysis. The experiment was repeated four times, using fresh D_2O and with newly prepared Ti cathode. The rise in temperature measured here is considered as the resultant temperature of heating and cooling of the electrolytic solution. At the current densities used for the electrolysis D_2 and O_2 are produced [$Cl_2(g) + 2e \rightleftharpoons 2Cl^-$ (aq), $E^0 = 1.358$ V (ref. 19), and $O_2 + 4D^+ + 4e \rightleftharpoons 2D_2O$, $E^0 = 0.815$ V (ref. 19)].

We now analyse the rate of temperature increase, considering it as arising from Joule heating or from a chemical reaction (table 1). A combination of the

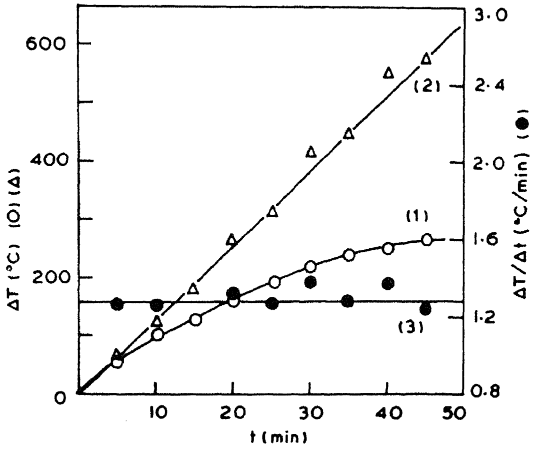

Figure 2. Plot of ΔT vs t during the electrolysis of D_2O at Ti cathode and Pt anode. Curve 1 is the ΔT vs t plot with no cooling correction applied to the measurement. Curve 2 is the ΔT vs t plot with the cooling correction as described in the text. Curve 3 is the $\Delta T/\Delta t$ vs t plot (after the cooling correction) for the electrolysis of D_2O.

Table 1 *Rationalization of power balance in the electrolysis of D_2O*

E_{app} 2.58 V, i_0 0.033 A cm^{-2}, cell current 0.50 A	
Case I:	
Power consumed in the electrolysis of D_2O	= 0.77 J s^{-1} (W)
Balance of power remaining	= 0.52 J s^{-1} (W)
Expected rate of temperature change	= 0.52/116.18 J s^{-1}/J°C^{-1} = 0.0045°C s^{-1} = 0.27°C min^{-1}
Experimentally measured rate of temperature change (after cooling correction)	= 1.30°C min^{-1}
Case II:	
a) Exothermic adsorption of D_2 on cathode	
Heat produced by exothermic reaction*	= 120 kJ mol^{-1}
Rate at which charge is passed (100% current efficiency for the electrolysis)	= 0.50 C s^{-1}
Moles of D_2 produced by electrode reaction	= 2.59 × 10^{-6} mol s^{-1}
Expected power from the exothermic reaction	= 0.30 J s^{-1} (W)
Expected rate of temperature change	= 0.15°C min^{-1}
b) Exothermic reaction due to $2D_2 + O_2 \rightarrow 2D_2O$	
Heat generated by recombination reaction	= 285.83 kJ mol^{-1}
Expected power for the number of moles of D_2 produced in 1 sec	= 0.73 J s^{-1} (W)
Expected rate of temperature change	= 0.38°C min^{-1}

These calculations were done by accurately measuring the potential difference across the cell and the current through the cell. Rate of cooling was measured by raising the electrolytic solution to the same steady state temperature as during electrolysis, turning off the electrolysis and monitoring the temperature over time. Mass of D_2O = 22.00 g, mass of Ti and Pt plates = 18.77 g, mass of glass beaker = 17.12 g. D_2 adsorption on Ti will increase the weight of the cathode very marginally (in mg) which is neglected in the calculation.

For a hypothetical situation in which all the input power is consumed for Joule heating, the expected rate of temperature change is 0.68°C min^{-1} (no electrolysis occurring).

Experiments conducted with distilled water showed a rate of change of temperature of 0.26°C min^{-1}, which is substantially less than the value obtained with D_2O.

*This value is taken from Bailar, J. C., Emelius, H. J., Nylhom, R. and Trotman, A. F., (eds), *Comprehensive Inorganic Chemistry*, Pergamon Press, London, 1973, vol. 1, p. 51. The ΔH refers to the difference between metal–deuterium bond energy (E_{MD}) and the deuterium dissociation energy (E_{DD}).

two cases is also considered. For the Joule heating in the electrolysis of D_2O, first the power required to split D_2O into its constituents should be subtracted from the total power that is fed into the electrolytic cell. In other words, out of the total potential difference applied to the cell, E_{app}, one part will go to establish the thermoneutral potential of 1.54 V, caused by the heat of reaction and the heat of dissociation of deuterium and oxygen, i.e.:

$$D_2O + e \rightleftharpoons D_{\mathrm{ads}} + OD^- \qquad (1)$$

$$D_{\mathrm{ads}} + D_2O + e \rightleftharpoons D_2 + OD^- \qquad (2)$$

$$D_{\mathrm{ads}} + D_{\mathrm{ads}} \rightleftharpoons D_2 \qquad (2a)$$

and

$$4\,OD^- \rightleftharpoons D_2O + O_2 + 4\,e. \qquad (3)$$

The balance of power is considered as being utilized for Joule heating, which produces the temperature change in the cell. This scheme is shown in table 1. For the second case, two possible exothermic processes occurring in the cell are considered: (i) adsorption of D_2 on Ti, which is estimated to release 120 kJ mol^{-1}, and (ii) recombination of D_2 and O_2 in the electrolysis, which releases 285.83 kJ mol^{-1} (this calculation assumes that D_2 is not adsorbed during electrolysis).

Pauling[5] attributed the excess enthalpy in the Fleischmann and Pons experiment[1] to the formation of Pd deuteride and its subsequent decomposition. The decomposition of deuteride is explosive. As Ti has 0.72 orbital per atom of the orbitals vacant in the outer shell[5], this could be used for deuteride formation. Our experiments do confirm, Ti lattice expansion and generation of a cracking noise[2] during the electrolysis due to adsorption of D_2.

The data presented in table 1 allow an enthalpy estimate to be made of the exothermic chemical reaction. Subtraction of the expected excess temperature in case II from the measured excess temperature in case I yields 0.87°C min^{-1} (a) or 0.65°C min^{-1} (b). To obtain this temperature change we should expect an exothermic reaction of 0.32×10^6 J mol^{-1} with D_2 adsorbed on the metal, or 0.38×10^6 J mol^{-1} for the case when the difference in temperature is solely due to the chemical reaction with negligible adsorption of D_2 occurring. Under the experimental conditions used here this is inconceivable by ordinary processes.

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19 September 1989; Revised 5 October 1989

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PLASMA REDISTRIBUTION IN LOW LATITUDE IONOSPHERE DURING INTENSE SPREAD F CONDITIONS

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IT has been shown that during periods of intense night-time equatorial spread F the columnar electron content is reduced at the equatorial regions and increased at tropical latitudes. The generation of equatorial F region irregularities on certain nights is associated with intensification of equatorial plasma fountain in the evening hours due to the continuation of horizontal electric field in the eastward direction even during the post-sunset period.

Scintillations of radio waves from stars or from artificial satellites received at equatorial latitudes have been known to be associated with spread F irregularities[1-4]. The discovery of scintillations even at GHz range of radio waves[5-7] at equatorial latitudes has generated keen interest in studies concerning radio wave scintillations and cause and effects of F region plasma irregularities. Unfortunately, during periods of intense equatorial spread F, most of the techniques to monitor the ionospheric plasma density distribution fail due to strong reflected or scattered echoes from these irregularities or due to violent fluctuations of the amplitude of trans-ionospheric radio waves from geostationary satellite. *In situ* measurements of plasma density by satellite-borne probes have shown large bite-outs up to three orders of magnitude in plasma density because of the plasma bubbles associated with spread F over the size scales of 10 to 200 km[8]. Therefore, no measurements have been reported of the temporal variation of background plasma density or of electron content at equatorial regions during intense spread F.

With the cooperation of the Environment Research Laboratory, Boulder, USA and the Physical Research Laboratory, Ahmedabad, India, a sophisticated receiving station was set up at Ootacamund, India, to record the amplitudes of various carrier and side bands of the radio beacons from geostationary satellite ATS-6 then situated at 35°E longitude. The system and measurement techniques have been described by Davies *et al.*[9] The amplitudes of all the signals were recorded separately on two orthogonal aerials into digital tapes at intervals of 0.1 s during October 1975–January 1976. To this day these data remain unique for equatorial latitudes and have not been repeated at any other low latitude station.

During intense spread F conditions, the analogue records of Faraday fadings of the received beacons are obliterated due to rapid and violent fluctuations of the signal amplitude, rendering the measurement of ionospheric electron content impossible during these conditions. Using the 0.1 s digital data of beacon received at Ootacamund, Bhattacharyya and Rastogi[10] have shown that during periods of intense amplitude scintillations, the Faraday phase fluctuation for 140 MHz is negligible although the quadrature components from which the Faraday rotation angle is derived show large fluctuations. Thus, using digital data, it has been possible to compute the true Faraday rotation of the 140 MHz signal and thereby compute the ionospheric electron content (N_F) over the equator even during intense scintillation events. Using the amplitude data at interval of 0.1 s the scintillation index S_4 for every 15 min is defined as the root mean square deviation of the power of the received signal divided by the mean power. The Physical Research Laboratory had also organized the measurement of N_F at a number of low and tropical latitude stations during the same period using Titheridge-type polarimeters. The data were recorded on analogue strip charts. During the study, scintillations on Faraday phase fluctuations were not recorded at Ahmedabad or Patiala. The N_F values were available at these stations during the periods when strong amplitude scintillations were observed at Ootacamund. These data were supported

Table 1 *Geographical locations at various stations studied*

	Station coordinates			Sub-ionospheric coordinates		
Station	Lat. °N	Long. °E	Magnetic dip °N	Lat. °N	Long. °E	Magnetic dip °N
Kodaikanal	10.2	77.5	3.5	—	—	—
Ootacamund	11.4	76.7	6.0	10.6	72.9	4.4
Ahmedabad	23.0	72.6	34.0	21.5	69.4	31.6
Patiala	30.4	76.4	45.0	28.2	72.1	42.3

by vertical incident ionosonde observations at Kodaikanal and Ahmedabad. The geographical locations of the stations whose data are used in the present paper are listed in table 1. With respect to the equatorial F layer anomaly belt, Kodaikanal/Ootacamund were close to the trough, Ahmedabad was close to the crest and Patiala was well outside the belt. The N_F or S_4 data were computed for every 15 min intervals for the period October 1975 to January 1976. Based on S_4 (140 MHz) data at Ootacamund two groups of days were selected (i) when strong scintillations were recorded, and (ii) when practically no scintillations were observed. The lowest limit of no scintillation on chart corresponded to digitally derived $S_4 = 0.04$. The average temporal variation of S_4 was computed separately for these two groups of days for the hours 1600–2400 h for the entire period October 1975–January 1976. Similarly the mean temporal variations of N_F at Ootacamund, Ahmedabad and Patiala as well as the minimum virtual height of the F layer and the per cent occurrence of spread F at Kodaikanal were computed for the two groups of days. The resultant curves are shown in figure 1.

First, the mean value of S_4 on no scintillation days varied between 0.03 and 0.04 corresponding to peak fluctuations of 1/3 dB, for any of the hours between 1600 and 2400 h. On the other hand, the mean S_4 on scintillation present days was around 0.03–0.04 up to 1815 h after which it started to increase reaching a value of 0.40 by 2015 h and remained at the same level up to midnight. The individual values of S_4 ranged from 0.02 to 0.90.

It was found that no spread F was observed on no-scintillation nights. The mean per cent occurrence of spread F on the scintillation nights was zero up to 1800 h after which it started increasing to 100% at 2130 h and decreased to a value of 60% by midnight. Good correlation was seen in the mean curves of spread F and scintillations.

The temporal variation of $h'F$ at Kodaikanal started increasing from 220 km at 1600 h to 260 km between 1800 and 1900 h after which it started decreasing towards midnight. This increase of $h'F$ during the evening hours was due to the increasing zenith distance of the sun causing relatively faster decay of ionization in the lower levels of the F region, giving an impression of uplifting of the layer. On the scintillation-present nights also $h'F$ was 220 km at 1600 h, continued to increase faster reaching a value of 300 km at 2000 h. It is to be noted that $h'F$ on these days was not only higher

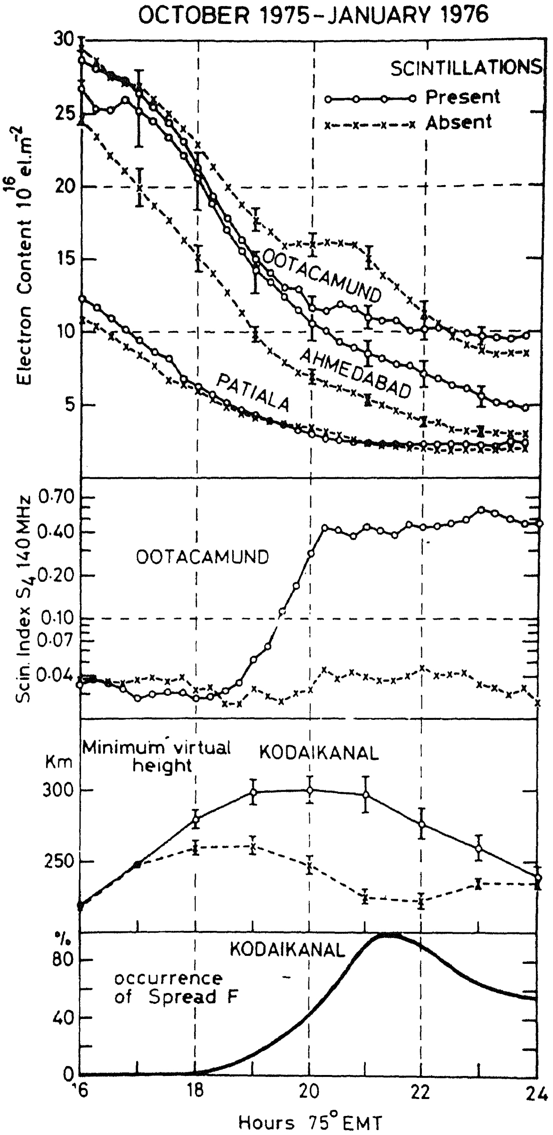

Figure 1. Temporal variations of minimum virtual height of F layer ($h'F$) at Kodaikanal, scintillation index S_4 at Ootacamund, ionospheric Faraday electron content at Ootacamund, Ahmedabad and Patiala averaged for two groups of days when scintillations were present and another when scintillations were absent at Ootacamund for the period October 1975 to January 1976.

but the peak value was delayed with respect to the same on scintillation-absent days. These effects are to be interpreted as due to genuine uplifting of the F layer besides the effects due to the decay of ionization at low levels.

Referring to the curves showing temporal variations of N_F at Ootacamund it is seen that N_F was lower on scintillation-present nights even since 1700 h when $h'F$ also indicated a faster rise. The value of N_F at 2000–2100 h, when S_4 reached the maximum value, was 11.5×10^{16} electrons m^{-2} on scintillation-present days compared to 16.3×10^{16} electrons m^{-2} on scintillation-absent days. The difference of N_F at Ootacamund on two groups of days was insignificant after 2200 h.

The values of N_F at Ahmedabad were significantly higher on days when scintillations were present at Ootacamund. This effect continued throughout the period 1600–2400 h. Thus the effects of scintillation source at the equatorial regions produced a decrease of N_F at equatorial and an increase of N_F at tropical latitudes. The difference in N_F between two groups of days became insignificant at Patiala, a station well outside the equatorial F region anomaly belt.

This phenomenon is very analogous to the development of F_2 region anomaly during the daylight hours as the consequence of equatorial plasma fountain resulting from the action of eastward electric field on the northward magnetic field causing upward lift of plasma over the equator followed by transfer of plasma to tropical latitudes guided by the lines of earth's magnetic field. Rastogi[11] has shown that spread F and scintillations at Huancayo are observed only on the nights when the horizontal/vertical electron drifts measured at Jicamarca showed the continuation of eastward electric fields even well after sunset. This electric field causes uplifting of the whole F region during sunset hours.

Numerically solving the time-dependant plasma continuity equation including the effect of ionization production by solar ultraviolet radiation, loss through charge exchange and transport by diffusion, $\bar{E} \times \bar{B}$ drift and neutral winds, Anderson and Klobuchar[12] showed that the post-sunset enhancement of $\bar{E} \times \bar{B}$ drift is primarily responsible for the post-sunset increase in ionospheric electron content at a station close to the crest of equatorial F region anomaly belt.

The present results conclusively show that the generation of equatorial spread F irregularities is associated with intensification of latitudinal plasma density anomaly which requires a strong electric field. The hierarchy of the phenomena is the continuation of the eastward electric field in the evening beyond the sunset hour, causing the anomaly to develop. With the generation of sharp gradient at the base of the F region after sunset the eastward electric field generates $\bar{E} \times \bar{B}$ gradient drift instability, sometimes referred to as bubbles. The buoyancy effects associated with the Rayleigh–Taylor instability mechanism extend the irregularities to greater altitudes over the equator. Together with the plasma fountain, the irregularities are lifted up and later diffuse to higher latitudes creating an equatorial belt of high incidence of spread F occurrence at low latitudes, analogous to the daytime belt of the bite out of F_2 layer critical frequencies. This idea fits in very well with the latitudinal belt of high occurrence of spread F observed by ground ionosondes[13] or by satellite-borne ionosondes[14].

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PETROCHEMICAL BEHAVIOUR OF AN ACIDIC–BASIC CONTACT ZONE

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THE study area is about 30 km south of Purulia, West Bengal and represents the northeastern extension of the Archaean plateau of Chotanagpur granite gneiss[1,2]. Rocks in this area comprise mica schist, amphibolites, different types of granitoid rocks, migmatites and intrusive dolerites[3]. These intrusives are considered to be a part of Newer Dolerites of Singhbhum and range in age between 1100 and 950 m.y.[4] The thermal history of these intrusives is complicated. The intrusives have undergone metamorphism in some places. Petrochemical changes in the characters of the country rock as well as those of the contact zone due to dolerite intrusion have been studied and presented here.

The area is flat, with gentle undulations. The host rock of the area, tonalite gneiss, is overlain unconformably by the Gondwana sediments[5], and grades imperceptibly into granodiorites and quartz-diorite towards north and south respectively (figure 1). Two phases of structural deformations are distinct. The first phase has isoclinally folded the country rock with an east–west trending axial plane. A second episode of folding produced minor open folds on the foliation plane, with axes plunging at 45° towards 305°.

Tonalite consists of plagioclase, microcline, quartz, biotite, hornblende, sphene, epidote, allanite, apatite, opaques and muscovite. The plagioclase feldspar appears to be of two generations: the younger generation ones are generally fresh, subhedral and vary in composition from An 27 to An 42 (andesine), whereas the early generation plagioclase (more calcic, An 30 to An 51) are commonly untwinned, small-sized, anhedral and slightly altered. Albite rim along the contact of plagioclase and microcline and allanite at the core of epidote are present. Biotite was in some places found to have replaced hornblende, as evidenced by the concomitant development of other minerals such as sphene, epidote, apatite and quartz.

The central part of the intrusive sill is medium-

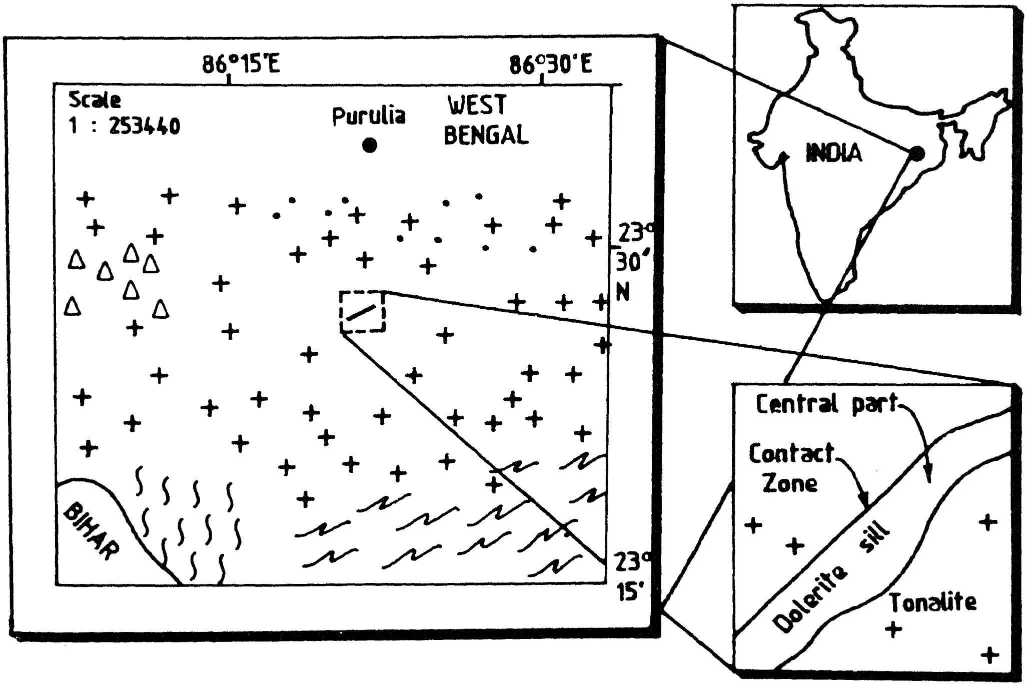

Figure 1. Geological map of the study area. + +, Tonalite gneiss; ••, granodiorite; ⌇⌇, mica schist; ∿∿, quartz diorite; △△, amphibolites; ⁄ dolerite.

grained, and has cloudy and twinned plagioclase (An 63), and shows typical intergranular and ophitic texture. Fine, equant hornblende grains are found coronated along their outer periphery, by pyroxene and almandine garnet, in that order (figure 2).

Rocks along the contact zone are fine-grained and show evidence of intense crushing. Occurrence of crushed pyroxene (augite) within the bent polygonized plagioclase can be interpreted to have been mechanically derived from the peripheral crushed pyroxene granules during deformation. Scapolite might have been formed due to the alteration of plagioclase feldspar under a condition of shearing and temperature difference.

Chemical analyses show (table 1) a decrease in SiO_2, TiO_2, Fe_2O_3, FeO, K_2O and P_2O_5, and an increase in Al_2O_3, MnO, CaO and Na_2O in rocks from the contact zone compared to tonalite gneiss, indicating basification of the acidic country rock as a result of intrusion. Release of Na, Ca and Mg from the dolerite body possibly facilitated the formation of more hornblende and augite in the contact zone. Corona structure (figure 2), formed by reaction between already crystallized phenocryst and intrusive dolerite melt; high content of SiO_2, TiO_2, FeO, P_2O_5 and K_2O in tonalite; and similar type of enrichment of SiO_2, MgO and CaO in dolerite of the study area compared to their average compositions[6] (table 1) indicate thermochemical mobilization of elements across the contact zone.

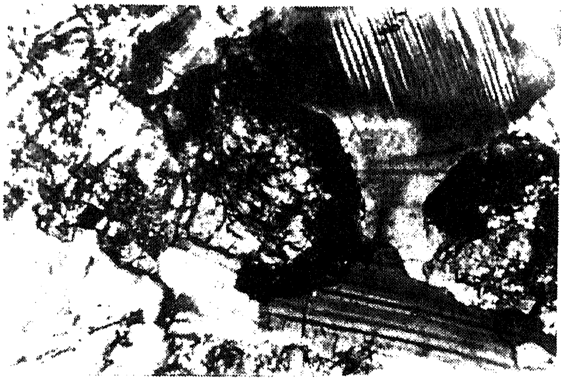

Figure 2. Corona structure with garnet rim enclosing the granular pyroxene grains (crossed × 40).

Table 1 *Chemical analyses of some major elements (wt %)*

Oxides	A	B	Bl	C	D	Dl
SiO_2	68.29	66.94	61.52	53.07	51.81	50.18
TiO_2	0.42	1.53	0.73	0.42	0.36	1.14
Al_2O_3	15.81	13.72	16.48	13.76	15.14	15.26
Fe_2O_3	0.64	1.51	1.83	1.31	1.26	2.86
FeO	2.30	4.05	3.82	3.46	4.89	8.05
MnO	0.03	0.04	0.08	0.10	0.19	0.19
MgO	0.99	2.65	2.80	9.37	8.81	6.78
CaO	4.34	4.21	5.42	15.89	14.97	9.24
Na_2O	4.35	2.18	3.63	2.29	2.26	2.56
K_2O	1.97	2.46	2.07	0.26	0.24	1.04
P_2O_5	—	0.28	0.25	0.03	0.03	0.27

A, Granodiorite; average of 3 samples. B, Tonalite gneiss; average of 6 samples. Bl, Average composition of tonalite, (Cox *et al.*[6]). C, Contact zone; average of 5 samples. D, Central part of the sill; average of 4 samples. Dl, Average composition of dolerite, (Cox *et al.*[6]).

The temperature of metamorphism (almandine–amphibolite facies) must have been appreciably high, as indicated by the presence of higher amount of garnet and its coexistence with hornblende[7], and preservation of relict minerals and texture[3]. However, the younger metadolerites experienced comparatively short-duration metamorphism, which commenced at the peak of the regional metamorphic episode.

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AN EMPIRICAL MODIFICATION TO HIGASI'S EQUATION FOR THE EVALUATION OF DIPOLE MOMENT OF A POLAR SUBSTANCE IN A NON-POLAR SOLVENT

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SEVERAL methods are available[1-6] for the evaluation of dipole moment of a polar molecule in a non-polar

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*For correspondence

Permittivity ; refractive In

solvent from static dielectric measurements in dilute solutions. According to the method of Higasi[5] the dipole moment is given by

$$\mu = B\,(a_0 - a_\infty)^{\frac{1}{2}} \tag{1}$$

where

$$B = \frac{1}{2(\varepsilon_1 + 2)} \sqrt{\frac{27\,MKT}{\pi N d_1}}, \tag{2}$$

a_0 is the slope of the graph ε_{12} vs W, a_∞ the slope of the graph n^2_{12} vs W, ε_{12} the dielectric constant of the solution, n_{12} the refractive index of the solution, W the concentration of the dilute solution, M the molecular weight of the solute, K Boltzmann's constant, N Avogadro's number, T the absolute temperature in Kelvin, d_1 the density of the solvent, and ε_1, the permittivity or dielectric constant of the solvent.

To employ the method the concentrations are to be so chosen that they conform to the idea of dilute solutions, to which Debye's theory is applicable. For such low concentrations the variation in values of

Table 1 *Dipole moment determination by the Higasi method and by using the proposed equation for polar compounds*

Compound	a_0	a_∞	μ by Higasi method (D)	μ by proposed equation (D)
*Methyl anthranilate	1.150	0.220	1.14	1.20
*Butyl isovalerate	1.970	−0.116	1.75	1.67
*Butyl formate	3.100	−0.260	1.78	1.68
5-Bromopyrimidine	4.000	0.100	1.89	1.87
*Amyl benzoate	2.058	0.057	1.89	1.88
*Methyl benzoate	2.198	0.094	1.90	1.90
*Isopropyl benzoate	2.501	0.061	1.93	1.91
*Ethyl cinnamate	2.720	0.216	2.01	2.05
*Isobutyl benzoate	2.546	0.028	2.03	2.01
*Dimethyl malonate	4.700	−0.100	2.42	2.35
*Methyl salicylate	4.423	0.239	2.42	2.43
*Diacetyl adipate	2.932	0.130	2.45	2.45
Indole	6.820	0.100	2.51	2.48
*Dipropyl adipate	2.934	−0.107	2.55	2.45
*Ethyl lactate	5.960	−0.225	2.60	2.50
*Dimethyl phthalate	4.008	0.115	2.63	2.62
*Ethyl salicylate	4.782	0.114	2.67	2.65
*Isobutyl salicylate	4.089	0.045	2.69	2.65
**n*-Amyl salicylate	3.950	0.091	2.73	2.71
2,3-Dimethylindole	6.860	0.470	2.92	2.95
2,5-Dimethylpyrazine	11.430	0.500	3.08	3.08
3-Methylpyridazine	13.650	0.500	3.15	3.14
Ethyl 5-ethoxy-3-methyl-indole-2-carboxylate	5.330	0.620	3.26	3.39
Ethyl 1-cyanomethyl-5,7-dimethyl-3-phenyl-indole-2-carboxylate	5.080	0.170	3.26	3.25
Ethyl 1-cyanomethyl-5-chloro-3-methyl-indole-2-carboxylate	7.270	0.290	3.31	3.31
1,2-Dimethylindole	9.300	0.360	3.45	3.45
2,5-Dimethylindole	9.500	0.320	3.49	3.48
3-Phenylindole	10.450	0.910	3.72	3.80
5-Bromoindole	9.180	0.440	3.96	3.98
Ethyl 3,5-dimethyl-indole-2-carboxylate	8.640	0.500	4.02	4.05
N-(2-methyl-5-nitro) phenylsydnone	12.59	1.200	4.49	4.62
2-Acetylbenzimidazole	17.140	−0.500	4.56	4.40
N-(3,4-dimethyl)phenylsydnone	34.170	1.530	4.83	4.84
2-(1-Hydroxyethyl)-benzimidazole	26.000	5.000	5.22	5.69
2-Acetyl-1-methylbenzimidazole	29.000	0.530	6.30	6.23
N-(2,5-dimethyl)phenylsydnone	16.410	1.000	7.03	7.10
N-(4-methoxy)phenylsydnone	46.400	3.940	8.12	8.26

Solvent: Benzene for compounds marked *. 1,4-dioxane for the rest.

refractive index from one concentration to another is generally found to be only in the fourth decimal place. Hence a very sensitive refractometer is needed for accurate measurement of n_{12}. A simple empirical modification of equation (1) is proposed, which eliminates refractive index measurement.

The equation proposed is

$$\mu = 0.98\, B \sqrt{a_0}. \qquad (3)$$

Employing a set-up of a crystal-controlled transistor oscillator using a crystal of 1 MHz and a very sensitive variable capacitor (0.250 pF in steps of 0.05 pF), the capacitance of the cell with and without a dilute solution of the polar compound in the non-polar solvent used was found. From the change of capacitance the static dielectric constant of the solution ε_{12} was calculated. This was repeated for five different concentrations. Refractive index was also measured for each dilute solution using the sodium D line in an Abbe refractometer at room temperature. From the plot of ε_{12} vs W the slope a_0 and from the plot of n_{12}^2 vs W the slope a_∞ were found. In the present work the slopes were evaluated using computer-aided curve fitting by the least-square method. Using (1) and (3) the dipole moments were calculated for several polar compounds. The results are given in table 1. The solvents used were benzene for some compounds and 1,4-dioxane for the rest. Most of the experimental data are taken from refs. 7–9.

It is clear from table 1 that a_∞ is small compared to a_0 and the value $(a_0 - a_\infty)^{\frac{1}{2}}$ is empirically found to be more or less equal to $0.98\,(a_0)^{\frac{1}{2}}$.

The proposed equation has been applied to a variety of polar molecules, whose permanent dipole moments range from 1.14 D to 8.12 D. The results obtained agree well with those by the Higasi method. The proposed equation gives equally accurate results but at the same time refractive index measurements are not needed. Thus the method can be used in any laboratory to find dipole moment of a polar molecule from measurements on dilute solutions in a non-polar solvent. The method does not require a costly refractometer but only a simple RF arrangement to measure change in capacitance.

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UNRECORDED PATHOGEN ON BAMBOO CAUSING BLIGHT IN INDIA

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Bambusa arundinacea (Retz.) Willd. is used for scaffolding in construction of buildings, in agricultural implements and in paper industries in India. Recently, a serious leaf blight was noticed in the forest nursery of the College of Agriculture, Dharwad. The intensity, calculated from per cent leaf area infected, was as high as 38.26%.

Infected leaves showed reddish to dark brown lesions with a grey centre, oval to elliptical in shape. Lesions were longitudinally distributed over the leaf lamina and measured 2–4 × 1–2 mm. Occasionally, they coalesced to give a blighted appearance. Culture of infected leaf bits on potato dextrose agar consistently yielded a species of *Exserohilum*. Colonies were pale greyish brown, later turning to dark brown. Conidiophores cylindrical, simple, geniculate, olivaceous brown, 2–6-septate, 40–180 × 4–8 μm; conidia acrogenous, elliptical or narrowly obclavate-rostrate, brown, thick-walled, subhyaline at the apex, usually straight, 1–15-septate, 29.6–130.0 × 8.0 × 25.0 μm. Cultural and morphological characters of the pathogen were found to agree with those of *Exserohilum halodes* (Drechsler) Leonard and Suggs anamorph of *Setosphaeria rostrata* Leonard. The culture of the fungus has been deposited in CMI, with accession No. IMI 315520.

A-week-old sporulating culture was successfully used to produce artificial infection on healthy bamboo seedlings. Typical symptoms were noticed on leaves 7 days after inoculation. Review of the literature revealed that *E. halodes* has been reported on several graminaceous hosts[1,2]. However, the present observation constitutes a new host record for *E. halodes* in India.

The authors are grateful to Dr Sivanesan of CMI, Kew, England, for identification of the pathogen.

17 February 1988; Revised 14 June 1988

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INHERITANCE OF A PUCKERED LEAF MUTANT IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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A plant with abnormal leaf characteristics was observed in groundnut variety OG 66–6–1. The plant, which was dwarf and compact, had puckered leaves (partially crinkled) with yellow stripes along the margin, with the exception of a few older leaves which had normal leaf phenotype. It had sequential flowering like the other plants in OG 66–6–1. The plant was harvested and progeny-rowed following the selection of puckered leaf plants in each generation till the phenotype was established. It was designated puckered leaf mutant (PLM). While such leaf characters may not be of any practical significance to groundnut breeders, morphological variants like this might be useful as marker traits in genetic studies.

The radiation-induced and/or naturally occurring mutants reported for leaf characters in groundnut are crinkle, curly, imperipinnate, lupins, brachytic, cup, willow, rusty, mosaic, multiple leaflets, corduray, flop, and Gujarat narrow leaf. There are also several chlorophyll-deficient mutants (xantha, chlorina, virescent, aureus, lutescens, etc.)[1–5]. However, PLM, with the above-mentioned characteristics, was not reported earlier in groundnut.

Parents and F_1, F_2 and backcross generations of the two reciprocal crosses involving normal leaf parents J 11 and MK 374 with PLM were grown in the 1984 rainy season at ICRISAT Center. Observations of leaf character were recorded for individual plants. The data were subjected to χ^2 analyses for testing the genetic ratio, after correcting for continuity following Yates[6].

The F_1 plants from both straight and reciprocal crosses of J 11 and MK 374 with PLM showed normal leaves.

The data for F_2 from these crosses were individually analysed for various F_2 ratios (table 1). A good fit to a 13:3 ratio of normal vs puckered leaves was observed in all crosses. The total and pooled χ^2 values were also nonsignificant ($P=0.01$) for a 13:3 ratio.

The BC_1 (F_1 backcrossed with PLM) showed a good fit for a 1:1 ratio of normal vs puckered leaves at 0.01 probability in all the crosses except (MK 374 × PLM) × PLM, for which the χ^2 value was significant at 0.05 probability (table 2). Similarly BC_2 (F_1 backcrossed to parent with normal leaf) produced only plants with normal leaves, except in

Table 1 *Chi-square test of various F_2 ratios of plants segregating for normal vs puckered leaves in two reciprocal crosses of groundnut*

	Leaf phenotype		χ^2			
Crosses	Normal	Puckered	3:1	13:3	15:1	9:7
J 11 × PLM	43	8	2.36	0.31	7.76**	16.33**
PLM × J 11	68	16	1.58	0.01	23.66**	20.82**
MK 374 × PLM	26	2	4.76*	1.77	0.34	15.24**
PLM × MK 374	35	5	3.30	1.03	2.66	15.86**
Total (4 d.f.)	—	—	12.00*	3.82	34.42**	68.25**
Pooled (1 d.f.)	172	31	10.24**	1.61	28.20**	66.91**
Heterogeneity (3 d.f.)	—	—	1.76	2.20	6.22	1.34

Significant at *$P=0.05$, **$P=0.01$.

Peanuts Leaf variants: Mutation

Table 2 *Chi-square test for 1 : 1 and 1 : 0 ratios of normal vs puckered leaves in the BC_1 and BC_2 generations of two reciprocal crosses of groundnut*

Crosses	Leaf phenotype Normal	Leaf phenotype Puckered	χ^2
Backcross 1 (1 : 1 ratio)			
(J 11 × PLM) × PLM	21	13	1.88
(PLM × J 11) × PLM	14	7	2.32
(MK 374 × PLM) × PLM	12	3	4.27*
(PLM × MK 374) × PLM	11	9	0.2
Backcross 2 (1 : 0 ratio)			
(J 11 × PLM) × J 11	16	1	1.06[a]
(PLM × J 11) × J 11	22	0	—
(MK 374 × PLM) × MK 374	10	0	—
(PLM × MK 374) × MK 374	25	1	1.04[a]

*Significant at $P = 0.05$.

[a] The χ^2 for testing 1 : 0 ratio was computed following $\chi^2 = m/n$, where n is the observed frequency in a normal class and m is the total.

(J 11 × PLM) × J 11 and (PLM × MK 374) × MK 374, in each of which one plant with puckered leaves was also observed. However, the χ^2 value in both of these BC_2 crosses was nonsignificant at 0.01 probability. The occurrence of a single plant with puckered leaves in BC_2 might be due to a chance mixing of seeds while processing the material.

The segregation pattern in F_2 of the two reciprocal crosses suggests that the normal leaf phenotype in groundnut is controlled by two pairs of genes, designated Nl_1 and Nl_2. For the development of puckered leaf character, the presence of the Nl_1 gene in recessive homozygous condition and the Nl_2 gene in dominant homozygous or heterozygous condition is essential. All other combinations will have normal leaves.

The backcross segregation pattern did not give strong support to F_2 observations when pooled analyses were done for a 1 : 1 ratio of normal vs puckered leaves. Frequency of puckered leaf plants was lower than expected in the backcross generation.

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A SIMPLE TECHNIQUE FOR POLLEN VIABILITY TEST

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A few techniques for testing pollen viability have been reported earlier[1–4]. Of these, the method using sugar-based agar medium appears to be of wider use in the case of many plant species. including *Crotalaria juncea*[5–7]. Though growth of pollen tubes was different in different media[5–7], there is also a report[8,9] that pollen of *C. juncea* germinated well, in a very weak solution of sugar (cane sugar, 0.1 M). The present report describes a very simple test for pollen viability.

The test included four species, viz. *C. retusa* L., *C. juncea* L., *C. sericea* Retz and *C. verrucosa* L. After anthesis the pollen was collected and dusted on clean, grease-free slides. Just after dusting, one or two drops of distilled water were added to the pollen mass and the pollen was spread carefully with the blunt end of a dissecting needle. After proper labelling each processed slide was placed on match sticks in petri dishes containing a small piece of moist blotting paper. The petri dishes were kept covered to maintain humidity. A parallel set of slides with pollen in cane sugar solution (0.1 M) was also set up. Pollen from both water and cane sugar medium was examined microscopically every hour. The material was stained with a drop of glycerine–acetocarmine mixture (1 : 1) and covered with a cover glass before observation. Pollen germination was recorded from ten samples and tube growth from 20 pollen grains picked at random.

Pollen germination in distilled water was almost as good as that in sugar solution (0.1 M) in the case of *C. retusa*, *C. juncea* and *C. sericea*, while the *C. verrucosa* there was a significant difference (table 1). *C. juncea* pollen tube growth in distilled water was significantly less than that in sugar solution (table 2). The above observations show that while distilled water alone cannot substitute for sugar solution or other sugar-based medium with regard to pollen

Crotalaria Sp.) Distilled water

Table 1 *Germination of Crotalaria pollen in cane sugar solution and distilled water under room temperature conditions*

Species	Sugar solution (0.1 M) Range (%)	Sugar solution (0.1 M) Mean (%)	Distilled water Range (%)	Distilled water Mean (%)	t
C. sericea	82.14–100	92.00 ± 1.80	75.60–94.59	86.00 ± 2.05	2.19*
C. retusa	92.30–100	96.03 ± 0.64	92.23–98.00	94.37 ± 0.55	1.95*
C. juncea	90.90–100	96.49 ± 1.06	89.65–100	94.91 ± 0.94	1.40^{NS}
C. verrucosa	91.89–100	94.45 ± 0.85	62.74–86.66	75.03 ± 2.28	7.96**

*Significant at 5%; **Highly significant; NS, not significant.

Table 2 *Crotalaria juncea pollen tube growth in sugar solution and distilled water*

Incubation time (h)	Sugar solution (0.1 M) Range (μm)	Sugar solution (0.1 M) Mean (μm)	Distilled water Range (μm)	Distilled water Mean (μm)	t
1	6–12	9.5 ± 0.38	4–10	7.5 ± 0.40	3.59
2	20–38	27.00 ± 0.96	6–20	12.5 ± 0.80	11.51
3	26–50	41.1 ± 1.61	12–22	17.1 ± 0.62	13.84
5	40–68	58.1 ± 2.12	22–48	31.00 ± 1.82	9.63
24	62–84	70.4 ± 1.58	24–50	32.4 ± 1.63	16.65

tube growth and development, it is sufficient for germination of pollen grains in some species, for which this method may be attempted.

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A NOTE ON THE ANEUPLOIDY IN *ARAIOSTEGIA PULCHRA* (D. DON) COPEL. AND *ASPLENIUM YOSHINAGAE* MAKINO VAR. *PLANICAULE* MORTON

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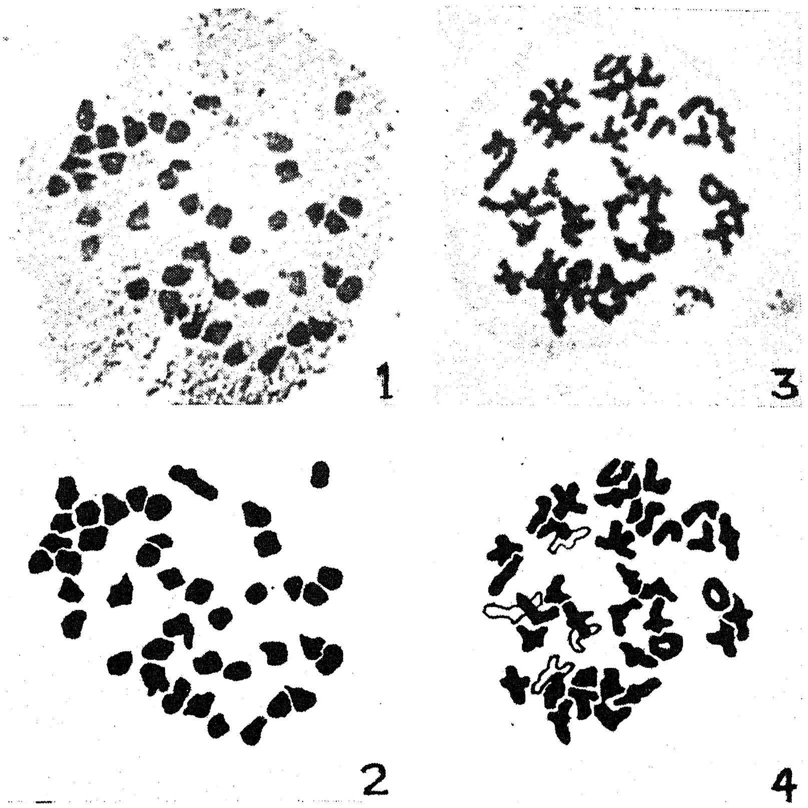
ASPLENIUM YOSHINAGAE var. *planicaule* (Aspleniaceae) and *Araiostegia pulchra* (Davalliaceae)[1] were collected from Damthong at 2200 m in South Sikkim and Aritar at 1200 m in East Sikkim. *A. pulchra* grows on tree trunks and *A. yoshinagae* var. *planicaule* at the foot of tree trunks under moist and shady conditions. Occasionally *A. pulchra* is found on damp rocks under trees on accumulated humus. Both ferns prefer epiphytic habitat.

Fixation of young sporangia was done in 1:3 acetic acid:alcohol on the spot. Acetocarmine (2%) was used as the staining reagent in squash preparations. At least 15 cells were analysed to obtain the chromosome number.

A. pulchra showed 41 bivalents at meiosis (figures 1 and 2). The earlier reports on it, however, are[2–4] $n=40$. *A. yoshinagae* var. *planicaule* exhibited 40 bivalents in spore mother cells (figures 3 and 4). This chromosome number, reported for the first time in the species, is comparable only to *A. unilaterale* Lam., where Bir[5] reported $n=40$ in plants from East Himalayas. *A. yoshinagae* var. *planicaule* differs



Figures 1–4. Squash preparations of **1,** *Araiostegia pulchra* $n=41$ (× 1000) and **3,** *Asplenium indicum* $n=40$ (× 1000). **2 and 4,** the corresponding diagrammatic representations.

taxonomically from *A. unilaterale* in having ascending rhizome, scaly stipe (fibrillose and often deciduous scales) and acute apex of pinnae. *A. unilaterale* possesses creeping rhizome, naked stipes and pinnae with obtuse apex. In *A. unilaterale* var. *udum* the pinnae have acute apex, as that of the present material[6]. Occasionally *A. yoshinagae* var. *planicaule* grows in association with *A. laciniatum* D. Don as an epiphyte on tree trunks and one is often confused for the other because of their morphological similarities.

A. laciniatum from the same habitat showed a haploid complement of $n=72$ in spore mother cells (unpublished work). Bir[5] also reported a tetraploid of this species from East Himalayas. *A. yoshinagae* var. *planicaule* is an interesting case of probable aneuploidy showing $n=40$. The genetic system here may be in a state of flux, which may result in the evolution of a new taxon. The present number $n=40$ is unusual for *Asplenium*. Kuriachen[7] reported $n=144$ (octoploid) in *A. indicum* from South India.

Araiostegia pulchra is a good example of aneuploidy showing $n=41$ as opposed to $n=40$, and might have been evolved from the latter. Panigrahi and Patnaik[8] have commented that the aneuploid methods of reduction or increase in the base numbers have played a major role in the evolution of ferns.

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FUNGICIDAL ACTIVITY OF TOLCLOFOS METHYL IN GROUNDNUT PLANT AND IN SOIL ON *SCLEROTIUM ROLFSII*

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TOLCLOFOS methyl (Rizolex) is a systemic fungicide (Sumitomo, Japan (Code No. S-3349), and Rallis, India). Chemically it is *O,O*-dimethyl-*O*-(2,6-dichloro-4-methylphenyl)-phosphorothioate and belongs to the organophosphate group of chemicals. It is reported to be effective against *Sclerotium rolfsii* and *Typhula*[1] spp. and also against *Rhizoctonia solani*[1-3]. The fungicide is also effective against *Thanatephorus cucumeris* (perfect state of *R. solani*)[4] and *Ustilago maydis*[5]. However, there is no information on translocation and persistence of the chemical in the plant system and its persistence in soil. Hence the present investigation was taken up.

Seeds of groundnut cultivar TMV-2 were treated with the fungicide at 1 and 2 g/kg of seed as dry mix. The treated seeds were sown in pots (30 cm diameter) each containing 3 kg of sandy loam soil and watered regularly. Untreated seeds were also sown to provide control plants. The plants raised from untreated and treated seeds were collected at regular intervals and presence of the fungicide was checked by bioassay of tissue extracts[6]. The plants collected were thoroughly washed with sterile distilled water, blotted with sterile paper towels, and separated into different parts depending upon the age of the plant:

Days after germination		Plant parts
1, 3	:	Whole plant
7	:	Whole plant, root and shoot
14, 21, 28, 35, 42, 49 and 56	:	Whole plant, root, shoot and leaves.

Five grams of plant material was ground thoroughly in a mortar with 10 ml of acetone. The ground material was filtered through cheesecloth, the filtrate collected in sterile glass vials (100 × 25 mm) and the acetone evaporated. The residue was taken up in 5 ml of sterile distilled water. This was added to 95 ml of molten potato dextrose agar and the agar was poured into petri plates (90 mm) after mixing. Tissue extracts

Table 1 *Characteristics of different soils employed in persistence studies*

	Red loam	Black loam	Sandy loam
Place of collection	Narakoduru (A.P.)	Agricultural College Farm, Bapatla (A.P.)	Agricultural College Farm, Bapatla (A.P.)
Previous crop	Coccinia	Groundnut	Groundnut
pH (1:2) Beckman pH meter (glass electrode) (Richards, 1954)	7.61	8.40	7.46
EC (mmho cm^{-1} Solubridge (Richards, 1954)	1.74	0.40	0.48
CEC (m.e./100 g soil) Neutral ammonium acetate method (Jackson, 1967)	9.40	50.00	7.60
Mechanical analysis (Piper, 1950)			
Sand %	64.97	43.10	84.45
Silt %	12.00	17.04	7.48
Clay %	18.00	39.86	6.94

Table 2 *Fungicidal activity of tolclofos methyl in groundnut plant following dry seed treatment*

Days after germination	*Radial growth of *Sclerotium rolfsii* (mm)															
	Whole plant				Root tissues				Shoot tissues				Leaf tissues			
	1000 ppm	2000 ppm	Control	Mean	1000 ppm	2000 ppm	Control	Mean	1000 ppm	2000 ppm	Control	Mean	1000 ppm	2000 ppm	Control	Mean
1	23.00	16.00	82.66	40.55	—	—	—	—	—	—	—	—	—	—	—	—
3	27.33	20.33	82.66	43.44	—	—	—	—	—	—	—	—	—	—	—	—
7	34.66	25.66	82.33	47.55	36.00	25.66	83.00	48.22	38.33	32.66	82.66	51.21	—	—	—	—
14	40.00	32.33	82.66	51.66	42.66	33.33	82.66	52.88	45.00	39.33	82.33	55.55	41.66	32.00	83.00	52.22
21	45.66	38.33	82.66	55.55	46.66	39.66	82.33	56.21	51.66	45.33	82.33	59.77	46.00	38.33	82.66	55.66
28	50.33	44.33	83.00	59.22	52.00	45.66	83.00	60.22	57.33	51.33	82.66	63.77	51.33	43.66	82.33	59.10
35	56.00	49.66	82.33	62.66	58.00	51.33	82.66	63.99	62.33	56.66	82.33	67.10	57.33	49.66	83.00	63.33
42	60.66	58.00	82.66	67.10	62.33	58.33	82.33	67.66	67.33	60.66	83.00	70.33	62.66	57.66	82.66	67.66
49	65.00	62.33	83.00	70.11	67.33	63.66	83.00	71.33	71.33	65.66	82.66	73.21	68.33	65.66	82.33	72.10
56	69.66	66.00	82.33	72.66	72.33	68.66	83.00	74.66	74.66	70.33	82.33	75.77	75.33	71.66	83.00	76.66
Mean	47.23	41.29	82.62		54.66	48.28	82.74		58.49	52.74	82.53		57.52	51.23	82.71	

	Concentrations (C)	Intervals (I)	C vs I	C	I	C vs I	C	I	C vs I	C	I	C vs I
S.E.	0.15103	0.27575	0.47762	0.1869	0.3053	0.5288	0.1926	0.3145	0.5447	0.1990	0.3040	0.5265
C.D. ($P=1\%$)	0.4024	0.7347	1.2725	0.5029	0.8215	1.4228	0.5182	0.8462	1.4656	0.5380	0.8220	1.4236

*Average of three replicates. —, not tested.

from untreated seed plants were used as control. All the petri dishes were inoculated with 7 mm mycelial discs of a 3-day-old culture of *S. rolfsii* isolated from infected groundnut plants (cv TMV-2). Radial growth of the fungus was measured after 72 h of incubation.

Persistence of tolclofos methyl in sandy loam, red loam and black loam soils was studied over 12 weeks. Characteristics of the soils are given in table 1. Samples of each soil type were passed through a 4.68 mm sieve after pounding. The soils were sterilized in an autoclave at 20 psi for 30 min twice on alternate days. One kg of sterile soil of each soil type was mixed with fungicide (1 and 2 g/kg of soil). The treated soils were kept in plastic beakers and sterile water was added regularly except on the day prior to soil sampling. Control soils were maintained without the addition of the fungicide.

Persistence of the chemical to a depth of up to 5 cm in the soils was studied by bioassay of samples collected using a cork borer. Fungicide present in the soil was extracted by taking 5 g of air-dried soil with 5 ml of acetone in glass vials (100 × 25 mm). The vials were then thoroughly shaken and left for 10 min to allow the soil to settle. The supernatant was transferred to sterile glass vials (100 × 25 mm) and the acetone was evaporated. The residue was taken up in sterile distilled water as before, and the bioassay carried out. Each treatment, including the control, was replicated thrice.

The data (table 2) show that tolclofos methyl was taken up by groundnut following dry seed treatment and was redistributed among different parts of the plant. All the extracts, prepared from plants on days 1, 3 and 7 and every week thereafter after germination, inhibited growth of *S. rolfsii*. The whole plant extracts from plants raised from seeds treated with 0.1% and 0.2% fungicide limited fungal growth to a greater extent than the corresponding root and shoot extracts. Extracts from older plants age (raised from treated seeds) were less inhibitory to the fungus than extracts from younger plants. Extracts prepared from fungicide-treated soils completely inhibited growth of *S. rolfsii*.

The inhibition of *S. rolfsii* by shoot and leaf extracts shows that the fungicide moved upwards in the plant system. The higher inhibition in the case of treatment with 0.2% tolclofos methyl may be due to higher initial uptake, and translocation and persistence of the fungicide in plant system. The lower inhibition by extracts from older plants (raised from treated seeds) may be due to dilution of the fungicide or degradation of the fungicide to non-fungitoxic products.

The better persistence of the fungicide in the treated soils may be due to slow rate of degradation by abiotic factors and absence of biological degradation.

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A NOTE ON THE SPONTANEOUS OILY LARVAL MUTANT IN SILKWORM, *BOMBYX MORI* L.

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DURING the course of our breeding studies in June 1988 some spontaneous oily mutant larvae were observed in a polyvoltine silkworm breed of *Bombyx mori* L. The larvae were highly oily and translucent when compared to normal creamy-white larvae (figure 1). The mutant larvae were separated and their performance was assessed and compared with that of the normal breed. After three generations the characters of the mutant strain were consistent; hence it is breeding true.

More than 25 different genes, located at different loci, are known to cause translucency of the skin. The integument of the silkworm larva is usually opaque, containing whitish crystals of urate[1]. But several mutants have become known which have

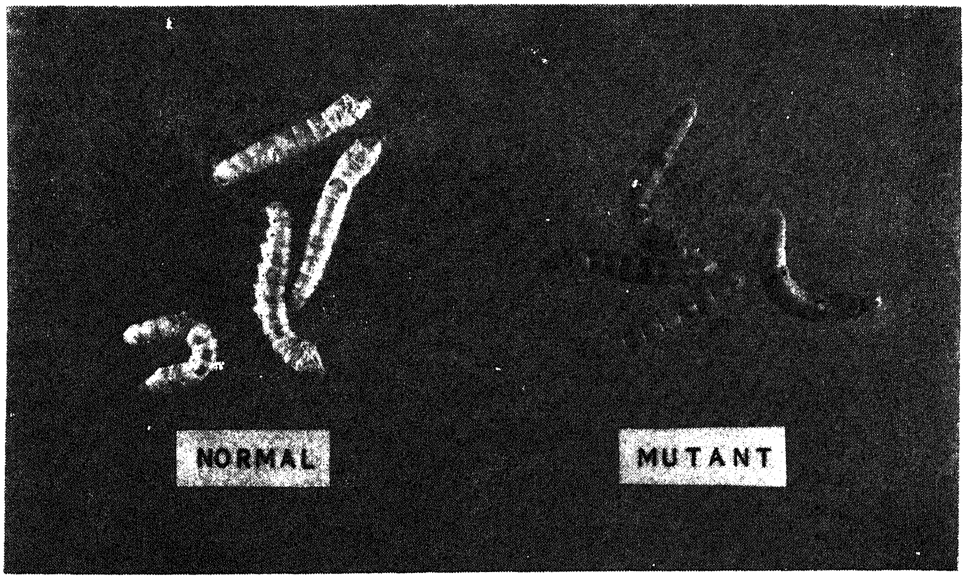

Figure 1. Normal and oily mutant larvae of silkworm.

translucent skin. The translucency varies according to the mutant strain, mainly depending on the quantity of urate in the epidermal cells[1,2].

In the present case, the oily character is very conspicuous and the mutant larvae were easily separated from the original batch. Of the 21 oily mutant larvae 11 were males and ten females. The mutant is identified as the *og* mutant, i.e. the Giallo Ascoli translucent of Sansaki[1], which is situated on chromosome 9 at locus 7.4. The translucency is of an extremely high degree. Translucent larvae are thought to have less ability than the normal type to retain uric acid in various organs and tissues of ectodermal origin. Translucency genes are more or less lethal. In the present case, the mortality was very high: of the 11 males only four larvae spun cocoons and of the 10 females only two spun cocoons. Sexual markings are very clear and it can be differentiated with the naked eye easily even after the third moult. All the four males and two females

Table 1 *Performance of oily mutant and normal polyvoltine silkworm*

	Generation	Larval duration (h)	Cocoon wt (g)	Shell wt (g)	Cocoon shell ratio (%)
Mutant	June–July '88	504	1.322	0.205	15.5
	Sept.–Oct. '88	510	1.302	0.200	15.4
	Nov.–Dec. '88	553	1.320	0.210	15.9
	Mean	522.33333	1.31467	0.20500	15.6
	SD	26.72838	0.01103	0.00500	0.26459
Normal	June–July '88	576	1.006	0.139	13.8
	Sept.–Oct. '88	594	1.087	0.150	13.8
	Nov.–Dec. '88	636	1.107	0.152	13.7
	Mean	602.000	1.06667	0.14700	13.76666
	SD	30.78961	0.05349	0.00700	0.05820
	t	3.3843*	7.8657**	11.6773**	11.7211**

emerged from the cocoons. Only one pair was recovered, as the other female was unable to pair with a male. All the eggs laid by the mated female moth were fertilized. In the subsequent generation the survival of the larvae and moths was observed to be satisfactory. Three generations (June–July, August–September and November–December 1988) of mutant and normal were compared and the data are given in table 1.

It is evident from table 1 that the cocoon characteristics of the mutant are superior to those of the normal. Another interesting feature is reduction in larval duration by 3 days even after three generations in the mutant strain. The improvement noticed in the mutant strain is 23% in cocoon weight, 39% in shell weight and 13% in cocoon shell ratio.

As the cocoon characters of the mutant are better than those of the normal and are breeding true, the mutant can be utilized in future breeding programmes to improve the economic characters of polyvoltine breeds.

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VIBRATION CHARACTERISTICS OF CANCER CELLS

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A cancer is a malignant mass of cells that divide uncontrollably to the extent that normal cells are deprived of nutrients[1]. In cancerous transformation cells lose, partially or completely, their normal surface properties[2]. It involves a radical reorientation in biosynthetic metabolism for cell growth and division[3]. Progressive increase in the permeability of the cell membrane[4], lack of contact inhibition of growth[2], and changes in membrane fluidity[5] (i.e. viscosity and rigidity of the cell membrane) are some of the well-known characteristic features of tumour cells. Cancerous growth reflects a disturbance in the cellular system. Various types of cell disorders are known to occur in man and other organisms[6], each of which has a characteristic frequency spectrum and amplitude of oscillations. The equations for the characteristic frequencies of oscillation of normal cells have been used to explain neoplasia in terms of altered characteristic frequencies.

The vibration characteristics of normal cells have recently been studied[7-9]. If one ignores the viscosity and relaxation parameter, the frequency equation of a normal resting cell can be written as[8,10]

$$\omega_n^2=\frac{T}{\rho_i b^3}\times\frac{n(n-1)(n+2)}{\left[1+\left(\dfrac{n}{n+1}\right)\dfrac{\rho_0}{\rho_i}\right]},\quad (n\neq 1), \qquad (1)$$

where n is a positive integer, T the interfacial tension, b the radius of the cell, and ρ_0 and ρ_i the density of the extracellular and intracellular fluid respectively. The surface and interface displacements are proportional to the Legendre polynomials[7] P_n^0 (cos θ). Rapid shape patterns continue throughout the cycle[2]. The variation of ω_n with ρ_0/ρ_i for different modes of oscillation of the cell is shown in figure 1. As the tension parameter $T/\rho_i b^3$ of the membrane changes (increased membrane fluidity is a characteristic of cancer and more rigid membranes are characteristic of aging), frequency of vibration changes (figure 2).

Changes in vibrational frequency from that of the normal resting cell[9] may be the cause of several metabolic diseases. The diameter of carcinoma cells[11] is 10–15 μm and the critical diameter at the time of division is estimated to be 12.6–18.9 μm (assuming that the volume doubles just before

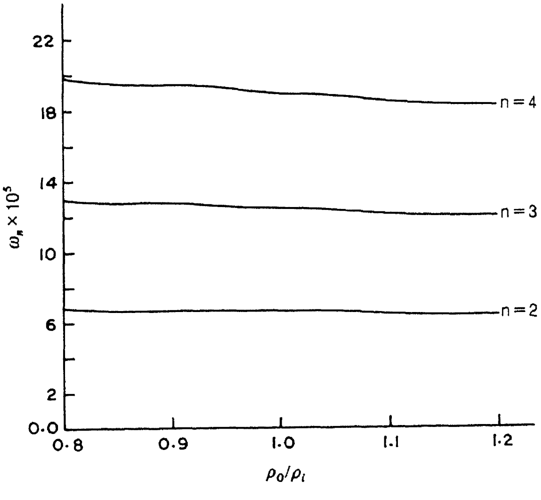

Figure 1. Frequency of vibration of a cell as a function of density ratio ($T/\rho_i b^3 = 9 \times 10^{10}$ s^{-2}).

vibration

mitosis). Abnormal vibration characteristics for different modes of oscillation of a cancer cell are shown in figure 3 (with time t). The period of the cell cycle has been divided into time intervals t_1, t_2, etc. The different phases of the cycle are also indicated in figure 3. The cells divide repeatedly (at a faster rate than do the corresponding normal ones[12]) without spending the normal time in interphase. The amount of time required for most cancer cells to pass through the S, G_2 and division phases of the cell cycle may be approximately the same as for normal cells but the G_1 period appears to be considerably shorter.

Mass transfer changes owing to changes in membrane fluidity (rigidity), growth patterns alter, and the cells divide at inappropriate times. Each type of cancer cell has a recognizably different abnormality in frequency spectrum which is different from that of the homologous normal resting cell[9] and is a characteristic of the severity of the malignancy. Cells become cancerous when normal G_1 periods become defective. The rate at which a cancer cell can divide depends partly on the severity of the defects in the controls[14].

One can conclude that alterations in interfacial tension due to changes in surface properties and

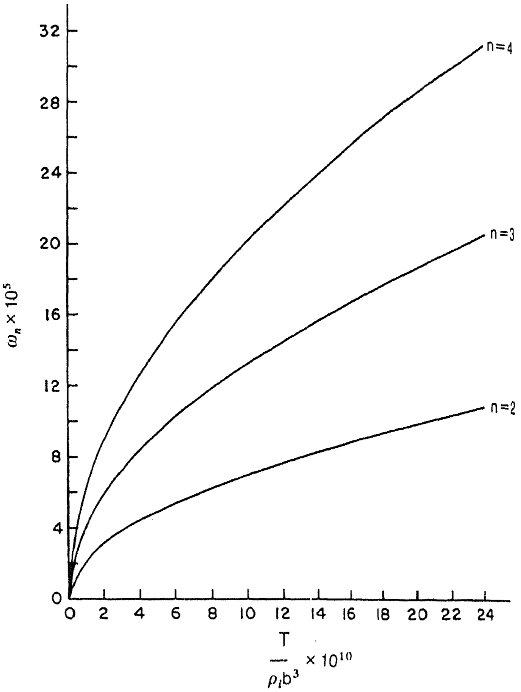

Figure 2. Frequency of vibration of a cell as a function of tension parameter $T/\rho_i b^3$.

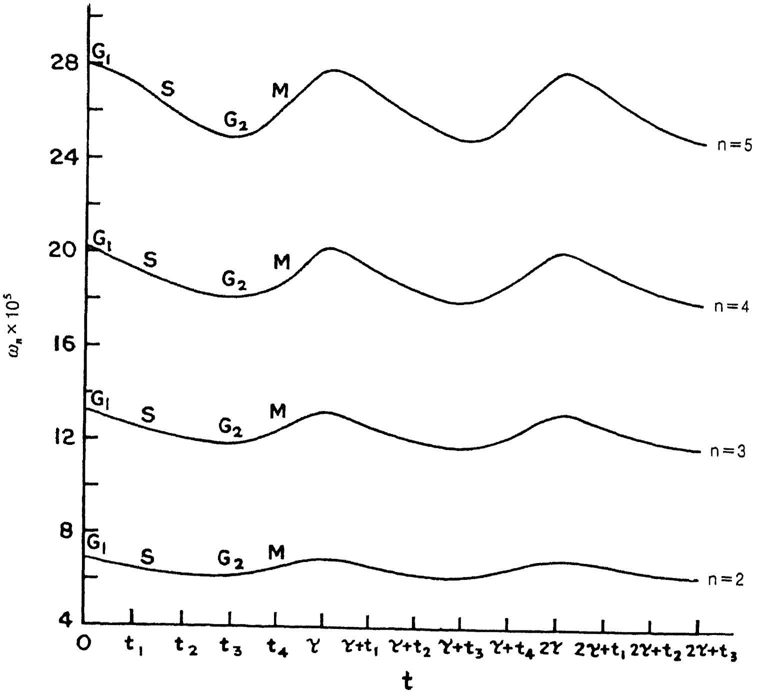

Figure 3. Plot of ω_n vs t for different modes of oscillation of a cancer cell ($\rho_0/\rho_i = 0.95$).

abnormal vibration characteristics are important factors in the establishment of the malignant state.

The author thanks Sri Chanchal De and Sri Sajal De for their help in the preparation of the manuscript.

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FECUNDITY OF MAYFLIES OF WESTERN GHATS OF PENINSULAR INDIA

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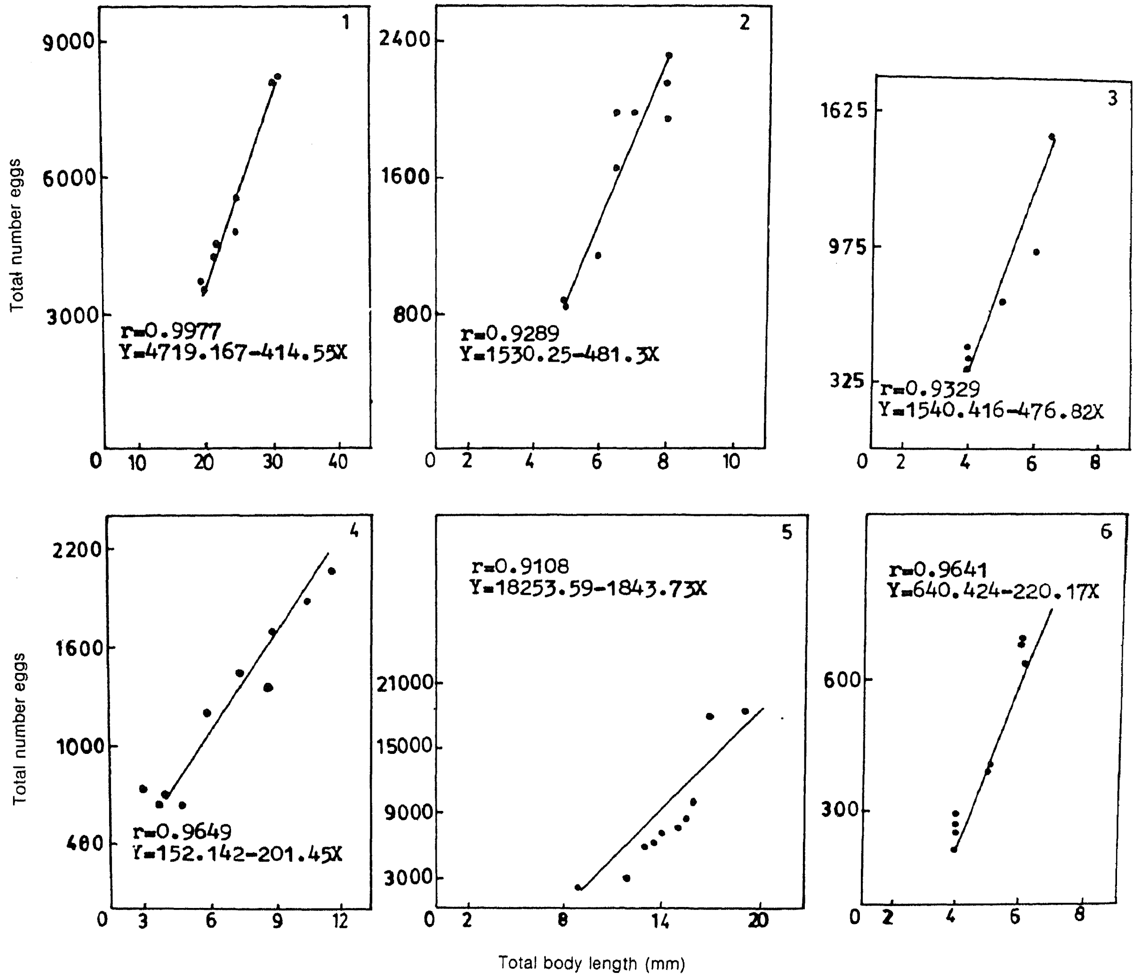
FECUNDITY is the total number of eggs produced by the female during her life span regardless of the fate of the eggs. For most insect groups, it is difficult to measure fecundity accurately in their natural environment because the eggs are continually maturing and are oviposited throughout the life span. Mayflies are excellent material for the study of fecundity under natural conditions. Adult mayflies usually live for only a day or two and all the eggs are produced prior to the subimago stage and the total potential fecundity can be determined accurately by examining subimagos, imagos that have not oviposited, and even last instar nymphs[1].

Mayfly fecundity has been correlated positively with body length[1–5]. A more meaningful calculation for making comparisons between species would be the relationship between egg production and unit body length. In the present study, six families of Ephemeroptera were chosen and the relationship between their egg production and unit body length was analysed. Eggs from the abdominal and thoracic body cavities of the last instar (with darkened wing pad) nymphs of mayflies were removed to a Sedgwick rafter and counted. The relationship between fecundity and body length was statistically analysed.

The correlation between the number of eggs produced by six families of Ephemeroptera and their body length is presented in figures 1–6. The average number of eggs per mm of body length in the six families ranged from 201 to 1843. The data of Clifford and Boerger[1] for Bigory River mayflies of Canada, of Hunt[2] and Britt[3] for Ephemeridae, and of Minshall[6] for Heptageniidae were 137–222 eggs/mm, 300–350 eggs/mm and 100–200 eggs/mm respectively.

In the present study the r value which ranged from 0.90 to 0.99 (highly significant) confirms the general view that fecundity increases with increasing body length of the nymphs. However, Minshall[6] found that beyond a certain size (10.5 mm) the number of eggs decreased with increasing size of the individual. This apparent decline in egg number with increasing body size (up to 31 mm in Ephemeridae) has not been observed in the present study.

Though the nymphs of Ephemeridae are the longest among the members of the six families under investigation, the rate of egg production is high (1843 eggs/mm) only in Heptageniidae. The ephemerids are burrowing and sandy forms whereas heptageniids are rheophilic and are restricted to torrential areas of rock-bottomed streams. This difference in ecology should account for production, in heptageniids, of more eggs as a compensatory measure for the loss of eggs washed away by torrents.



Figures 1–6. Correlation and regression values between the number of eggs and body length in **1**, Ephemeridae; **2**, Ephemerellidae; **3**, Caenidae; **4**, Leptophlebiidae; **5**, Heptageniidae; **6**, Baetidae.

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CRYSTAL STRUCTURE OF PUTRESCINE–GLUTAMIC ACID COMPLEX

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POLYAMINES are ubiquitous organic amines found in all living cells. Although their precise biological function is unknown, their importance in the life cycle of cells is reflected in the precise control mechanisms that have evolved to regulate polyamine concentrations[1]. Polyamines increase during infection of cells by pathogens and also upon induction of cancer[1].

During studies on the biophysical properties of belladonna mottle virus (BDMV), it was observed that the virions contain polyamines that are essential for the integrity of virion particle structure. The polyamines are most probably bound to the viral nucleic acid[2]. Replacement of these intrinsic ions by monovalent cations such as sodium, potassium or lithium leads to loss of particle stability at alkaline pH[3].

These and several other observations[4] suggest that the mode of interaction between polyamines and nucleic acids cannot be modelled solely on the basis of electrostatic interactions. In order to understand the mode of interaction of polyamines with other biological molecules, and to explore the potential of these biogenic amines to adopt to a variety of chemical environments, we have initiated X-ray crystal structure studies of different polyamines crystallized in a variety of biologically relevant chemical contexts. The crystal structure of a complex between L-glutamic acid and putrescine is described in this communication.

Crystals of a 2:1 complex of L-glutamic acid and putrescine were obtained from an aqueous solution by liquid diffusion of propanol. The crystals belong to the monoclinic space group $P2_1$, with $a=5.175$ Å, $b=22.889$ Å, $c=7.840$ Å and $\beta=93.43°$. The calculated density based on 2 glutamic acid and 1 putrescine in the asymmetric unit is 1.3 g cm^{-3}.

X-ray diffraction data were collected on an Enraf Nonius 4 circle diffractometer using CAD4 geometry. X-rays from a microfocus sealed tube equipped with a molybdenum anode ($\lambda=0.7107$ Å) were employed. A total of 1331 unique reflections were measured. The reflection intensities were corrected for Lorentz and polarization factors but not for absorption. During the data collection, standard reflections that were periodically monitored did not show any crystal decay.

The structure was solved by using the direct methods program MULTAN[5] and refined to an

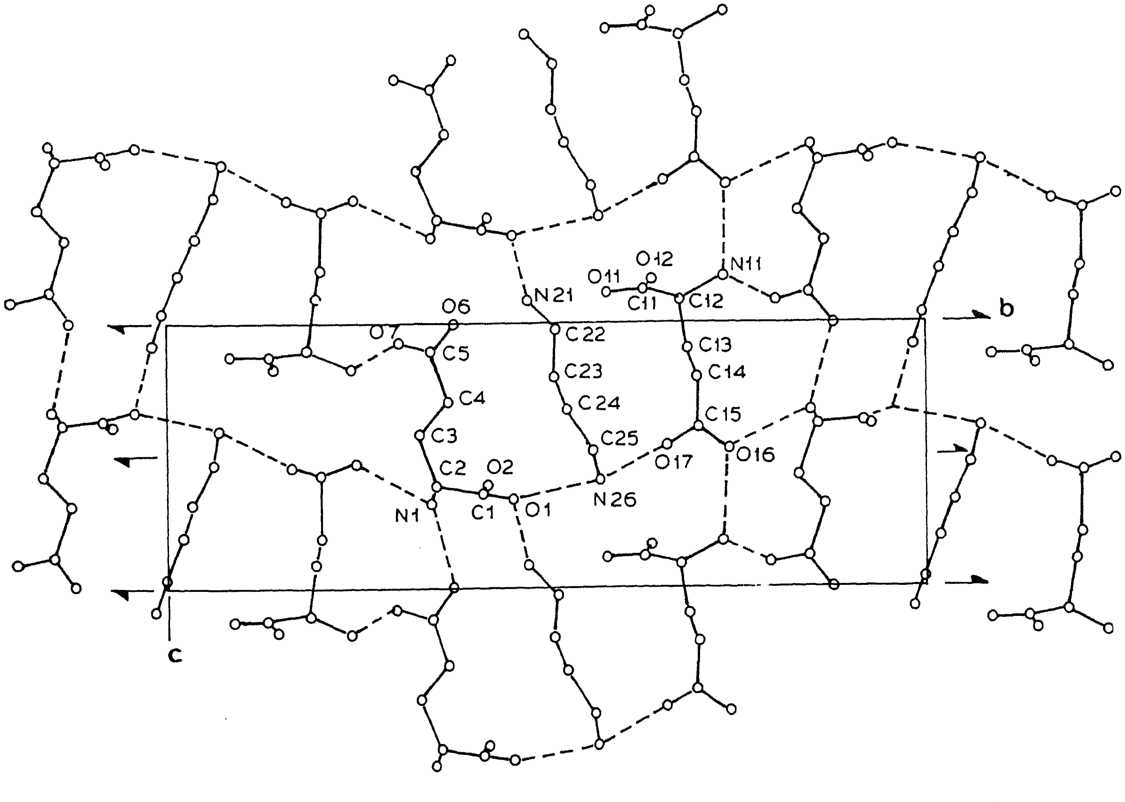

Figure 1. Projection of the structure of putrescine–glutamic acid complex on the *bc* plane.

Table 1 *Comparison of torsion angles of different glutamic acid forms*

Torsion angle (degrees)	α-form	β-form	Protonated form	Glu I	Glu II
N-C-C-O (1)	−50.2	−42.3	161.2	168.5	156.9
N-C-C-O (2)	130.3	141.2	−21.2	−10.9	−24.4
N-C-C-C	178.2	−51.8	−68.9	64.7	63.4
C-C-C-C	68.3	−73.1	−173.1	−179.6	174.8
C-C-C-O (6)	74.2	18.8	15.0	8.9	−53.0
C-C-C-O (7)	−104.6	−160.7	−167.0	−175.7	127.7

Glu I and Glu II are the two glutamic acid forms in the present structure.

$R = 5.1\%$ for reflections with $F_0 > 3\sigma(F_0)$, using a block diagonal structure factor least squares program. The calculations were performed on a DEC 1090 computer. During refinement the C, N, O atoms were refined with anisotropic temperature factors, whereas the hydrogens were associated with an isotropic temperature factor.

Examination of the final structure reveals that the two glutamic acid molecules in the asymmetric unit have two different conformations. Glutamic acid has been found to exist in α, β and a protonated form. In the α[6] and β[7] forms of L-glutamic acid, the α-carboxyl groups are protonated and a short hydrogen bond between the α-carboxyl and δ-carboxyl groups leads to a highly twisted side chain. In contrast, in the protonated form of L-glutamic acid hydrochloride[8], the side chain exists in an extended conformation. In the present case both carboxyls are charged and hence cannot be hydrogen-bonded amongst themselves. Accordingly the side chain conformation is closer, if not fully identical, to the protonated form. Table 1 lists the conformation angles of α, β and protonated forms of glutamic acid along with those of the two crystallographically independent glutamic acids in the present structure.

The glutamic acid amino groups hydrogen bond with both the main chain and side chain carboxyl groups. Table 2 gives a list of all the hydrogen bonding parameters. Figure 1 gives the projection of the packing diagram on the *bc* plane.

Table 2 *Hydrogen bonding pattern*

A	B	A–B length (Å)	H–A–B angle (degrees)	Symmetry		
N1	06	2.7044	2.85	*x*,	*y*,	*z*+1
N1	02	2.8096	24.72	*x*−1,	*y*,	*z*
N1	016	2.7543	15.04	−*x*,	*y*−0.5,	1−*z*
N11	07	2.8581	27.55	1−*x*,	*y*+0.5,	−*z*
N11	07	3.2964	20.73	−*x*,	*y*+0.5,	−*z*
N11	016	2.7882	10.54	*x*,	*y*,	*z*−1
N21	012	2.7048	8.17	*x*+1,	*y*,	*z*
N21	06	2.7619	13.16	*x*+1,	*y*,	*z*
N21	02	2.8042	5.18	*x*,	*y*,	*z*−1
N26	017	2.7708	10.40	*x*,	*y*,	*z*
N26	01	2.9585	4.45	*x*,	*y*,	*z*
N26	011	2.7737	20.38	*x*+1,	*y*,	*z*+1

Table 3 *Conformation angles of putrescine in the two structures*

Angle (degrees)	put.hcl	glu.put
N1-C1-C2-C3	−179.8	−177.1
C1-C2-C3-C4	−179.9	176.4
C2-C3-C4-N5	179.8	175.6

The conformation of putrescine in the complex is similar to those of its hydrochloride salts[9]. Table 3 gives the values of the conformational angles of putrescine from the two structures. It appears that the overriding influence in the crystal structure is the polyamine as the glutamic acid molecules have assumed conformations that are different from those found so far whereas the putrescine itself exists in its most favourable extended conformation. The observed molecular conformations satisfy the hydrogen bonding requirements of putrescine.

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A PRELIMINARY REPORT ON EARLY CELL TYPE-SPECIFIC ANTIBODIES IN *XENOPUS LAEVIS*

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In recent years much work has been done to find biochemical markers of developmental stages. Amphibian embryos are a focus of attention[1-3] in these studies. Such markers are temporally or regionally restricted in early amphibian development[4,5].

There are still very few cell type markers for early amphibian embryos. The aim of the present investigation was to obtain monoclonal antibodies against early germ layer-specific antigens. The monoclonal antibody 3D7 recognizes an antigen that generally appears to be localized close to the cell membrane and also all over ectodermal cells in the early development of *Xenopus laevis*.

Embryos obtained via artificial fertilization by standard procedures were staged according to Nieuwkoop and Faber[6]. Embryos were dejellied in 2% cysteine hydrochloride (Sigma), pH 8.0, and then cultured in MMR (pH 7.8).

Monoclonal antibodies were raised against a crude cell homogenate from late blastula stage of *X. laevis* embryos. Immune mouse splenocytes were fused with Sp2/O-Ag14 myeloma cells[7] using published procedures[8]. Hybridomas that gave positive reaction were subcloned twice by limiting dilution. Screening of hybridoma supernatants was by ELISA using a cell homogenate from embryos at blastula stage. Colour development was measured at 492 nm in a Titertek Multiscan. Hybridomas that gave positive reaction were further tested by immunocytochemistry. Monoclonal antibody 3D7 was used in the study.

Embryos were fixed in 0.1% glutaraldehyde/2% paraformaldehyde for 6 h at room temperature. After fixation embryos were washed in PBS, dehydrated with ethanol, and embedded in paraffin. Sections were cut on a Spencer 820 (Lameris, Utrecht) microtome, collected and dried at 37°C for 24 h. Sections were deparaffinated with xylol and ethanol. Incubation with 3D7 ascites fluid (1:1000 dilution) for 60 min at 37°C was followed by 3 washes in PBS before incubation with a 1:50 dilution of goat anti-mouse IgM(Fc)–FITC (fluorescein isothiocyanate, Nordic) for 60 min at 37°C. Slides were washed with PBS, mounted in PBS–glycerol (1:1), and examined under a Leitz fluorescence microscope.

In control experiments, cryostat sections of unfixed or methanol-fixed embryos were used. Antibody localization was the same, but the quality of sections was poor and the background immunofluorescence high. Of all fixation procedures tried, the glutaraldehyde/formaldehyde fixation combined with paraffin embedding gave the best signal-to-background ratio.

The temporal and spatial pattern of 3D7 immunoreactivity analysed by immunocytochemistry shows many interesting features. In the unfertilized egg, the antigen is not localized but evenly distributed over the cytoplasm (figure 1, a). By 30 min post-fertilization, however, a striking redistribution of the antigen occurs (figure 1, b): the antigen becomes localized in a broad band in the periphery of the egg. In the cleavage stages, the antigen remains localized in the outer periphery of the cytoplasm (figure 1, c and d). When more cell layers develop, this outer-peripheral localization is restricted to the peripheral cell layer (figure 1, d). The intracellular localization is also restricted to the peripheral cell layers in the late blastula and early gastrula stages (figure 2, a). When the embryo gastrulates, the antigen become localized in the blastopore region,

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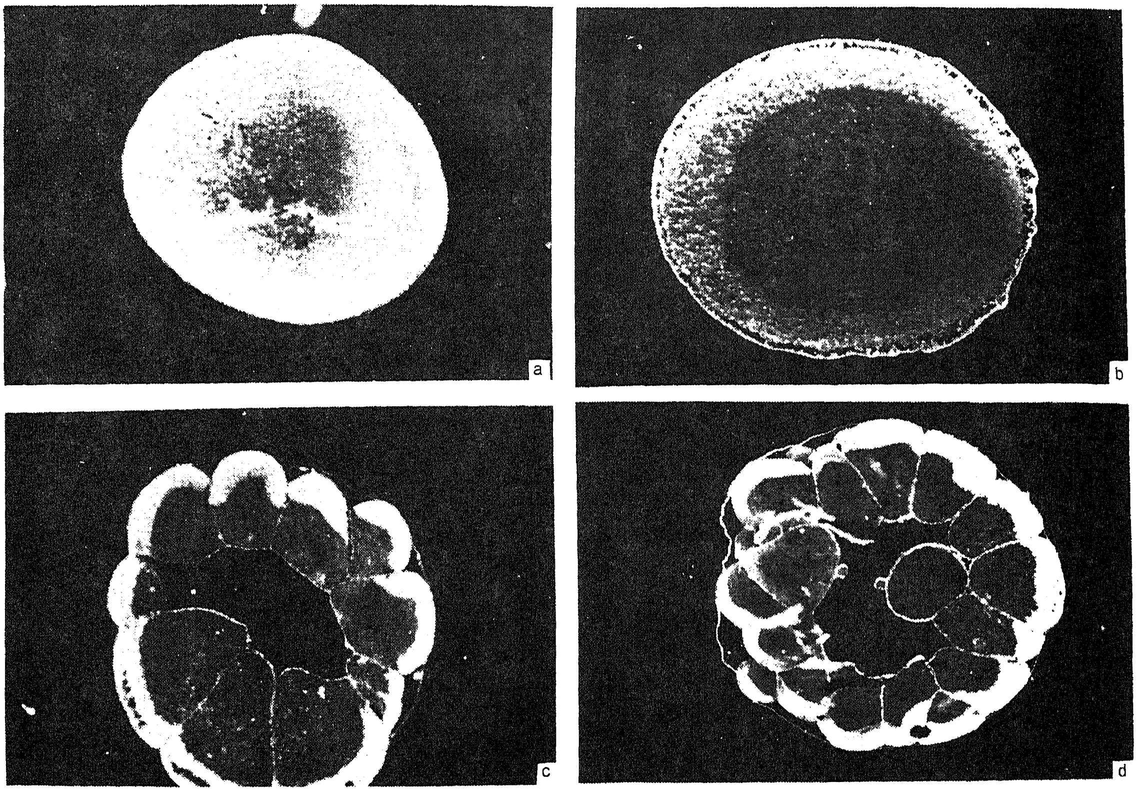

Figure 1. Distribution of antigen recognized by monoclonal antibody 3D7 in sections of early *Xenopus laevis* embryos. a, Unfertilized egg; b, fertilized egg, 30 min after fertilization; c, stage 5 embryo; d, stage 6 embryo (× 6.3).

both dorsally and ventrally (figure 2, b). Staining was found in the cells lining the blastopore and in the extracellular space between the two cell layers in the presumptive archenteron. No antigen was detected between the involuting mesoderm and the overlying ectoderm, the area in which both fibronectin[9] and laminin[10] accumulte during gastrulation. During neural stages, however, the antigen is found only in the ectoderm, the presumptive epidermis (figure 2, c). After stage 20 the antigen disappears completely. Up to the neurula stage the antigen is present both in the intracellular and extracellular matrix. The cell membranes are also stained and staining is observed between the individual cells (figure 1, b–d; figure 2, d).

There are various processes related to germ layer differentiation and pattern formation, which can only be followed with the help of cell marking experiments[6,11] using appropriate antibodies. The present work describes the appearance and distribution of an antigen recognized by the monoclonal antibody 3D7 for *X. laevis*.

The most notable facts concerning the localization of the antigen are:

i) The antigen is localized in a broad, bright, peripheral band immediately after fertilization.

ii) The localization changes dramatically during the earliest embryonic stages. Intracellular localization is restricted to the periphery of the embryo; extracellularly, it is found between all cells in the embryo. During gastrulation, the antigen also becomes localized in the blastopore region, inside the cells and between the cell layers that line the presumptive archenteron.

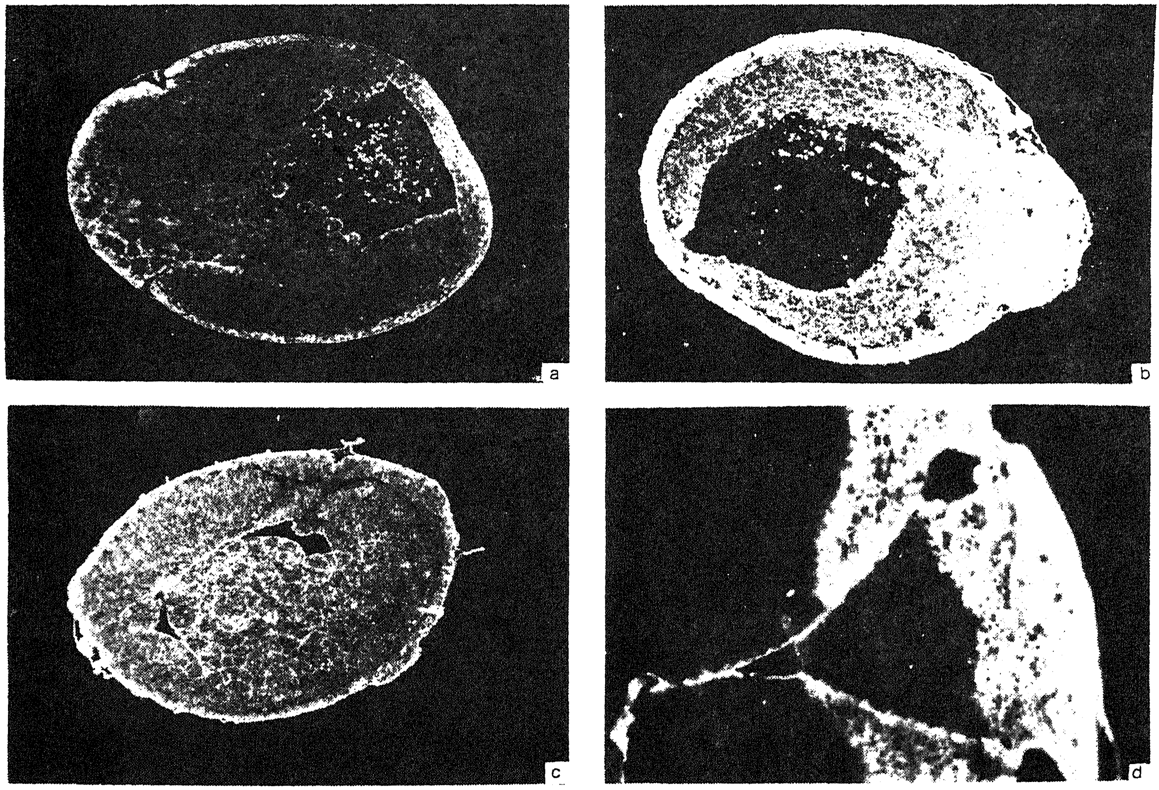

Figure 2. Distribution of antigen recognized by monoclonal antibody 3D7 in sections of early *Xenopus laevis* embryos. a, Stage 7 embryo; b, stage 8 embryo; c, stage 9 embryo; d, peripheral cell layer staining. (a, b, c, × 6.3; d, × 40).

The cause of the cyclic band pattern remains unclear. Biochemical characterization of the antigen is being carried out.

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BOOK REVIEWS

A Practical Approach to Sedimentology, by Roy Lindholm, (Published by Allan and Unwin, Exclusive distributors in India, Hindustan Book Agency (India), 17 U.B. Jawahar Nagar, Delhi 110 007) 1987, pp. 276.

The book gives a detailed description of sedimentary features and the methods used to evaluate them. It includes eleven chapters: (1) Description of sedimentary structures, (2) Analysis of sedimentary structures, (3) Primary grain fabrics, (4) Trace fossils, (5) Particle morphology, (6) Mineral identification using X-ray diffraction, (7) Grain size, (8) Sedimentary rock classification, (9) Heavy minerals, (10) Rock colour, (11) Environmental analysis. In general the mode of presentation is simple, direct and, where analytical methods are involved, instructional. A good point of the book is that every chapter is followed by examples and exercises to be completed by an aspiring sedimentologist.

Chapter 1 is a description of sedimentary structures classified as upper surface, internal structures, structures on the lower bedding surface and soft sediment deformation.

Practical applications of sedimentary structures in plaeo-current and basin analysis are given in chapter 2. Some statistical significance tests, along with sine and cosine, as applied to plaeo-current analysis, are illustrated, but some more advanced ones, such as those developed by Watson (Jour, Geol, 1966), might have been included.

Chapter 3 deals in a very elementary way with the problem of pebble, sand grain, intraclast and fossil orientation methods of analysis. Since orientation, especially of sand grains, plays such an important role in determining reservoir properties of oil- and gas-bearing classic rocks, a slightly longer chapter would have been very useful, especially to petroleum geologists.

Chapter 4 on trace fossils is well illustrated for identification and a scheme of classification is presented for environmental study purposes. This is a highly useful chapter.

The chapter on particle morphology is succinct and presents the classical concepts of shape definition and methods of analysis. Fourier shape analysis, developed recently, might have been included.

Chapter 6 (Mineral identification using X-ray diffraction) is extensive, gives introductory ideas of X-ray diffraction, and extensive 2θ tables for identification of a wide array of minerals occurring in sedimentary rocks. Another useful feature of the chapter is the presentation of X-ray diffractograms of important clay and carbonate minerals.

Chapter 7 (Grain size) and chapter 8 (Sedimentary rock classification) are self-explanatory and quite up-to-date. However, inclusion of loose grain size analysis by microscopic methods would have added to the list of currently available techniques.

In Chapter 9 (Heavy minerals) the study of accessory minerals is exhaustively treated. The chapter is useful especially for sedimentologists associated with the oil industry.

A distinctive feature of the book is the chapter on rock colour, rarely or not at all included in many textbooks on methods in sedimentary petrography. Rock colour, if intelligently analysed, is a useful indicator of sedimentary environments.

Environmental analysis (chapter 11) is the very objective of sedimentology, especially as applied in fossil fuel and mineral exploration. Straightforward methods are clearly illustrated and can be easily understood and applied to solve specific problems.

A lacuna in the book is the absence of chapters on model analysis of clashic rocks, staining of carbonate minerals in thin section, peel techniques, and study of insoluble residues. The information gained from these techniques would also immensely contribute to environmental analysis. The author, however, has his own reservations on the ommission of these topics in the text.

The text is well illustrated by block diagrams, line drawings and photographs. A researcher and/or student can easily follow the text. It is one of the few highly readable books available in sedimentology. In the reviewer's opinion, it must be a book on the shelf of every aspiring and professional sedimentologist.

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Annual Review of Nuclear and Particle Science, Vol. 38, 1988, pp. 819, (ed.) J. D. Jackson (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California 94306, USA), Price: USA \$ 49, Elsewhere \$ 53.

Over the years *Annual Review of Nuclear and Particle Science* has been making available to scientists valuable review articles on various topical subjects in the field of nuclear and particle science. The present volume continues that tradition, and contains excellent review articles covering frontline topics in the areas of nuclear physics, particle and high energy physics, nuclear astrophysics, accelerators and particle detectors.

While the original shell model of Mayer and Jensen provided the basic foundation for a theoretical understanding of the properties of nuclear states, the modern multiconfiguration shell model approach has been quite successful in providing a theoretical understanding of the complete spectroscopic characterization of nuclear levels. The status of the nuclear shell model, with particular reference to model spaces and effective Hamiltonians, Gamow–Teller and magnetic dipole matrix elements, electric quadrupole matrix elements, higher-multiple matrix elements and electron scattering form factors, has been comprehensively reviewed in an article in this volume. In recent years, the availability of energetic heavy ion beams has opened up a way to study the structure of fast-rotating nuclei, and these studies have revealed superdeformed shapes at high angular momentum in several regions of the nuclear chart. This subject, of much topical interest in nuclear physics research with heavy ion beams, has been reviewed in another article, which brings out the experimental and theoretical status of this field with particular regard to the superdeformed bands in ^{152}Dy and the cerium–neodymium region. In another article aspects of chaos in nuclear physics are brought out, which should be of much interest to those engaged in studies of statistical nuclear theory. The present status of manifestations of the D-state in light nuclei are reviewed in another article in the domain of nuclear physics. This article presents various evidences for the D-state in ^{3}He/^{3}H, ^{4}He and ^{6}Li, and also discusses the role of the D-state in capture reactions.

There are also review articles on subjects related to nuclear astrophysics. The article on the nuclear physics of the theory of supernovae is an excellent review of the present understanding of the nuclear equations of state (EOS) needed to describe superdense nuclear matter during the collapse of supernovae. After giving a general review of nuclear EOS, the article discusses the capture of electrons during the high-density nuclear phase reached in the collapse of supernovae to arrive at the high-density equation of state and concludes with a discussion on temperature dependence of EOS at high density. In another article, the exotic phases of hadronic matter and their astrophysical significance are discussed. The article also presents a review of the physics of strange matter, laboratory searches for strange matter, the astrophysics of strange matter, and the cooling of neutron/strange stars. This survey shows that low-energy quantum chromodynamics (QCD) remains a fertile area of research in particle physics and astrophysics. An interesting article on detection of cosmic dark matter includes discussions on weakly interacting massive particles, axionic dark matter and light neutrinos. Another article of much interest to both the high energy physics community and the astrophysics community reviews the subject of extensive air showers associated with discrete astrophysical sources. This article includes a discussion on observations of air showers of associated with Cygnus X-3 and other neutron star X-ray binaries and possible ultra-high energy γ-ray sources, and also various acceleration mechanisms.

There are two very informative review articles in experimental high energy and particle physics based on experiments with advanced high-energy accelerator facilities. One of these articles summarizes the first round of experiments at the low-energy antiproton ring (LEAR) at CERN. After a short survey on LEAR and its first round of experiments, the article discusses the antiproton–proton scattering and reaction, resonances in $p\bar{p}$ interactions antiprotonic atoms and antiproton–nucleus interaction. The other article reviews the results of many exciting and technically impressive experiments performed at the proton–antiproton collider at CERN. This article also discusses the implications of the above results for the standard model and for physics beyond the standard model. The main results on inclusive spectra and particle composition of hadronic jets in e^+e^- annihilation in the 10–40 GeV energy range are reviewed. The large amount of data on inclusive hadron production accumulated over the last years at the PEP, PETRA, CESR and DORIS machines are presented and discussed on the QCD-based models. A proper understanding of energy deposition in high-energy proton–nucleus collision is important to have a clear picture of the processes that take place during the collision. The review on this topic also points out that knowledge of energy loss and energy densities attained in proton–nucleus collisions is intimately connected to a review of transverse momentum and rapidity distributions.

There are several articles that pertain to the areas of theoretical particle and high energy physics. The subject of meson production in two-photon collision has been reviewed in one such excellent article, which concentrates on the contributions of two-photon physics to meson spectroscopy. It is known that the details of J/ψ production and decay have lent strong support to QCD. The subject of hadronic and radiative decays of J/ψ has been reviewed. Another article reviews the mathematical framework and theory pertaining to lie algebraic treatment of linear and nonlinear dynamics. The subject of electroweak symmetry breaking has been comprehensively discussed, with particular regard to unitarity, dynamics and experimental prospects.

We know that neutrino masses are taken to be zero in the standard model, and are aware that discovery of a nonzero mass for any of the neutrino species would have significant impact on particle physics theory. Thus, there being great interest in the accurate determination of neutrino mass, the review of the topic of direct measurement of neutrino mass should be of great value. The volume includes two articles explicitly on instrumentation and techniques. The radiofrequency quadrupole (RFQ) accelerator is a new type of linear accelerator specially suited to the acceleration of low-velocity ion beams and has served to be a very powerful injector for high-current accelerators. The development of RFQ accelerators and their applications are discussed. Nuclear particle detectors are the backbone of experimental nuclear and particle physics. The most important factor that determines their resolution is the electronic noise of the detector set-up. The low-noise techniques exployed in detectors are comprehensively reviewed in another article. Starting from the basics of the working of detectors, the article discusses origin and properties of noise, noise amplification and charge, and signal processing, with illustrative examples of proportional and drift chambers, semiconductor detectors and ionization chamber calorimeters.

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Voyager 2 and the world of Neptune

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After dramatic discoveries during its flybys of Jupiter, Saturn and Uranus, *Voyager 2* has now sent back breathtaking images of Neptune and its moons. The major discoveries in the Neptunian system include puzzling weather systems, broad sheet of ring material, peculiar offset and tilting of the magnetic dipole field, six new moons, and atmospheric composition and surface features of the biggest moon, Triton.

INTRODUCTION

THE *Voyager* odyssey is a truly remarkable example of the spirit of human exploration and adventure. *Voyager 2* and its sister spacecraft, *Voyager 1*, during their historic journey in the last 12 years, have provided an amazingly new view of the three giant planets, Jupiter, Saturn and Uranus, 40 of their moons, and their unique systems of rings and magnetic fields as well as interplanetary magnetic fields and particles, and ultraviolet sources among the stars. They have thus brought about a qualitative change in our understanding of the outer planets of the solar system. The Neptune encounter of *Voyager 2* represents a befitting culmination of these earlier spectacular scientific achievements.

Although described occasionally as being slightly 'arthritic' (due to a partial failure of the scan platform actuator during Saturn encounter, thereby limiting it to only low rate slewing) and a little 'hard of hearing' (due to the failure of the main command receiver shortly after launch and limited tone reception capability of the backup receiver), *Voyager 2* performed flawlessly during its most recent encounter with Neptune and its largest moon, Triton.

Astronomers had noted in the early nineteenth century that Uranus, discovered by William Herschel in 1781, was not faithfully following its predicted orbit around the Sun. They speculated that this might be due to the gravitational pull of an unknown planet. Two mathematicians, John C. Adams of England and Jean J. U. Le Verrier of France, independently predicted (in 1845 and 1846 respectively)[1] the location of the eighth planet. Le Verrier sent his predictions to Johann G. Galle of the Berlin observatory who began a search for the object immediately. Within an hour of his telescopic search, Galle discovered an unidentified disk, only about a degree away from Le Verrier's and a few degrees away from Adams' prediction. The next night he confirmed the discovery of the new planet.

Neptune is the eighth and currently the farthest planet from the Sun and the fourth largest. Pluto in its highly eccentric orbit moved closer to the Sun than Neptune in early 1979, and will continue in this relative position till early 1999. At a distance of nearly 4.8 billion kilometres from the Sun, Neptune completes one orbit in 165 years. The planet has an equatorial diameter of 49,600 km and a polar diameter of 48,600 km. It is nearly four times larger than Earth and only slightly smaller than Uranus. Yet it weighs about 10.5 Earth masses while Uranus weighs only 8.7 Earth masses. This is because Neptune has an average density of 1.66 g cm^{-3}, compared with the Uranian density of 1.19 g cm^{-3}. This suggests that Neptune has more rock and less hydrogen, helium and other gases than does its sister planet. The inner core consisting of a mixture of rocks and ices is estimated to have a diameter of 16,000 km. A mantle made up mostly of liquid ammonia, liquid methane and water ice surrounds the core. The Neptunian atmosphere contains hydrogen, helium, methane and ethane, the latter two gases being responsible for the absorption of red wavelengths from sunlight and giving a blue-green colour to the planet.

THE ERA OF *VOYAGER*

The *Voyager 1* and *Voyager 2* missions[2], meant to explore the giant planets of the outer solar system, were born out of a concept known as the 'Grand Tour', which essentially involved taking advantage of the geometric alignment of the outer planets in the late 1970s. This arrangement of the outer planets, viz. Jupiter, Saturn, Uranus and Neptune, which occurs once in about 176 years, would allow a properly pointed spacecraft to swing from one planet to the next without using large spacecraft propulsion systems. In other words, the gravity assist provided by each of the planets would bend the flight path of the spacecraft and increase its velocity sufficiently to enable it to reach the next destination. At the time of the last alignment, Neptune had not yet been discovered. The next alignment of the planets will occur about the year 2152.

Voyager 1, launched on a faster and shorter trajectory compared to *Voyager 2* on 5 September 1977, encountered Jupiter on 5 March 1979 and Saturn on 12 November 1980. Geometric constraints prevent a spacecraft making a close flyby of Saturn's moon Titan from making similar flybys of Uranus or Neptune subsequently. In taking *Voyager 1* to the proximity of Saturn's rings and Titan, its flight path was bent sharply northward out of the ecliptic plane. Presently *Voyager 1* is heading away from the solar system at an angle of about 35 degrees above the ecliptic. This spacecraft may cross the heliopause, the boundary between the Sun's magnetic influence and interstellar space, by the next century. *Voyager 2*, launched on 20 August 1977, encountered Jupiter on 9 July 1979 and Saturn on 25 August 1981. In order to enable *Voyager 2* to make flybys of Uranus and Neptune, it was targeted to a suitable point at Saturn that would automatically take the spacecraft to these outer planets (figure 1).

At Jupiter *Voyager 1* and *Voyager 2* discovered active volcanoes on the satellite Io, thin rings of dust encircling the planet, and four new moons. At Saturn *Voyager 1* determined the composition of the large Saturnian moon Titan. Titan's atmosphere was found to be primarily of nitrogen, but also containing simple organic compounds that might have evolved into living organisms if Titan was not so cold. Several moons were discovered. Saturn's rings were found to be dynamic, with thousands of tiny wave-like features, perhaps electrically charged dust particles levitated above the ring plane. Wind velocity at the cloud-tops was measured at about 1600 km/h, about twice that on Jupiter. At Uranus *Voyager 2* found a strange magnetic field with a corkscrew-shaped tail extending millions of miles into space. It was

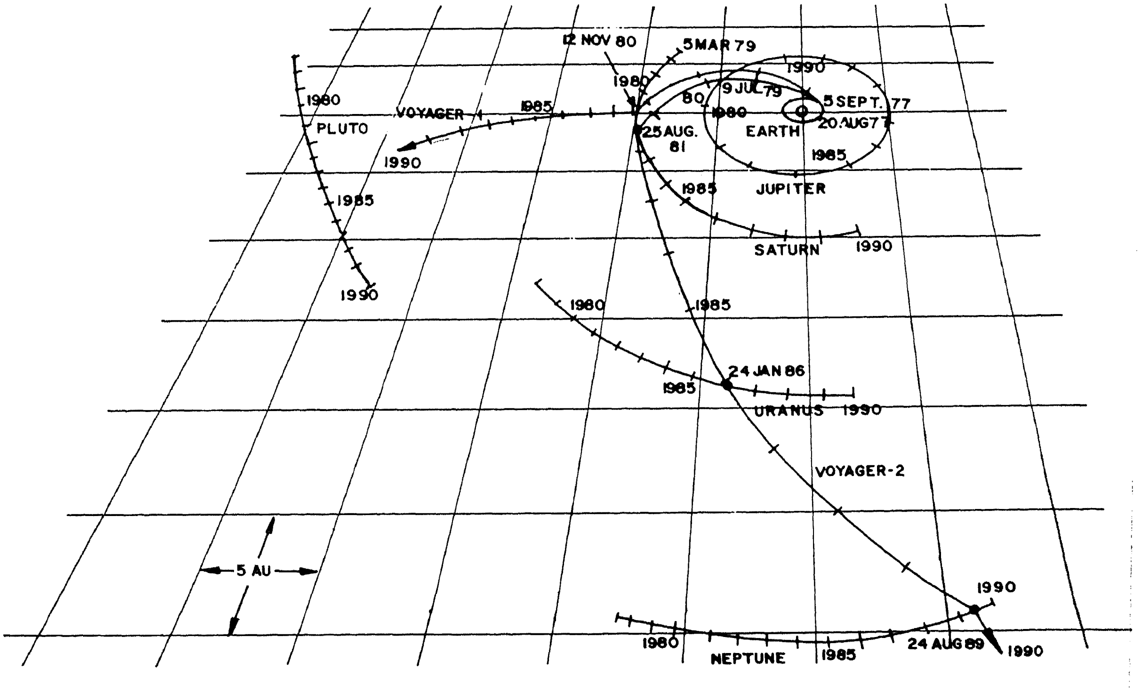

Figure 1. Trajectories of *Voyager 1* and *Voyager 2* with the dates of their encounters with planets.

Engineering adventure

Journey into Space: The first thirty years of space exploration, by Bruce Murray. W. W. Norton, New York, 1989, 381 pp., $19.95.

Bruce Murray is a professor at the renowned California Institute of Technology (Caltech). Just a few miles away is Jet Propulsion Laboratory (JPL), which started as a missile contractor for the US Army and is now owned and operated by Caltech. JPL became well known for its design of the *Explorer* satellite, which signalled the entry of the US into the space age. JPL is now a contractor for NASA, the US National Aeronautics and Space Administration. It has conceived, built and managed many spacecraft, including *Voyager 1* and *Voyager 2*. Bruce Murray was director of JPL when *Voyager 1* and *Voyager 2* were launched in 1977.

This book is the story of the remarkable deeds of a man and an organization, of their dreams and their struggles. Bruce Murray's dream has now become spectacular reality with *Voyager 2*'s rendezvous with Neptune.

We are told by the many reviews of the book in the American press that it is an inspiring book. Murray describes in great detail the working of JPL. He describes how NASA was never comfortable with the creative and independent JPL, which had the academic Caltech culture and eluded many governmental controls. Murray gives us a close view of the battles he fought and his courageous attacks of short-sighted government policies. To give an example, NASA had declared that the Space Shuttle should be the US's only launch system. Murray disagreed—not only because the Space Shuttle programme would take away most of the available funds—and insisted on development of alternative systems. The setback to the US space programme in the wake of the *Challenger* disaster and the inquiry into the disaster showed up the error of NASA's policy.

These are but a small part of a magnificent book, one that is full of engineering adventure. Murray's account of the rescue of a nearly failed spacecraft so that it could complete its mission reads like a Boys' Own adventure story to be read to be believed.

Murray tells us how he fell in love with Mars, which he first studied in 1960. Carl Sagan and he founded the Planetary Society, which now has a hundred thousand amateur astronomers as members. Now, Murray champions an international expedition to Mars in the next century.

Journey into Space is a story only a visionary and a pioneer can write, and one that must be read by anyone with even the slightest spirit of adventure.

S. R.

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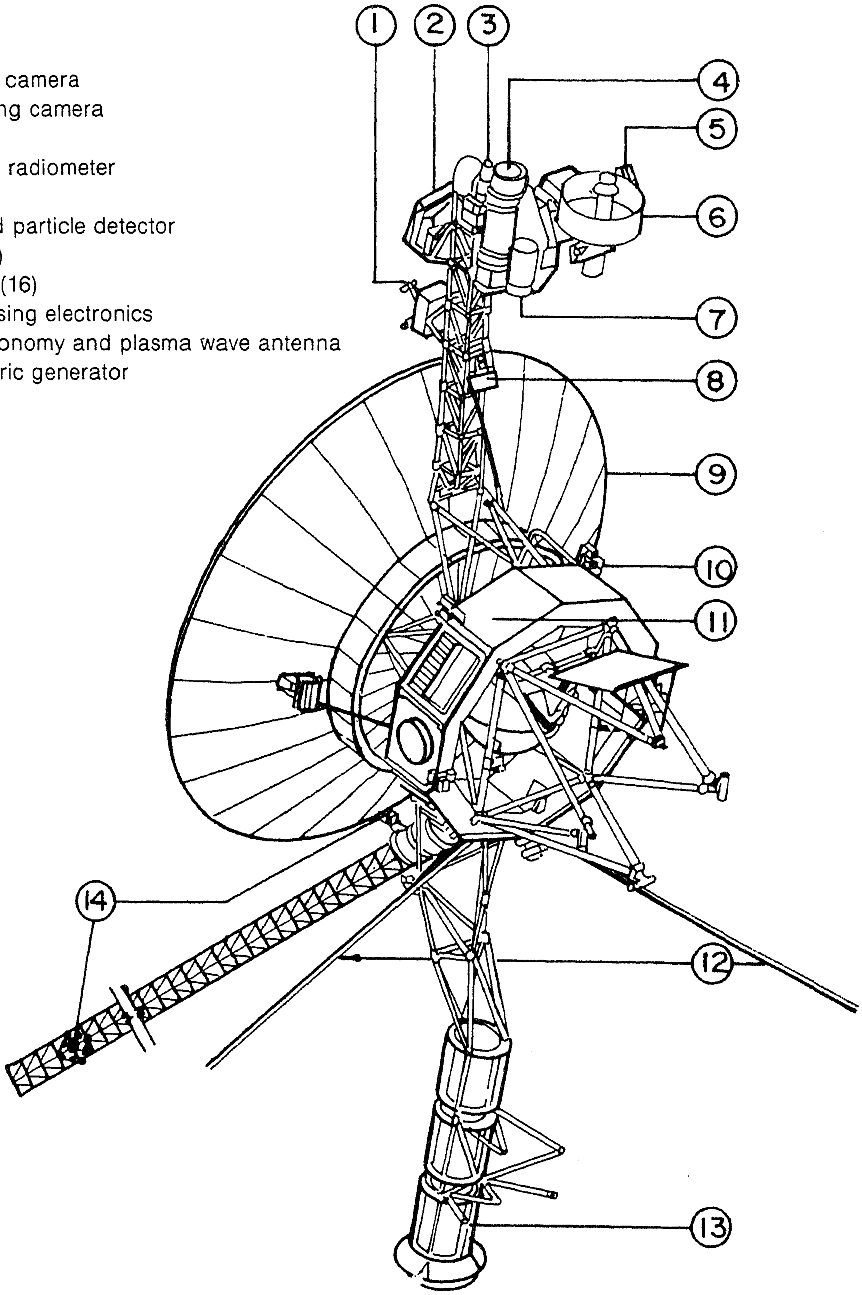
inferred from such information that electrically conductive atmospheric layers must exist deep beneath the visible atmosphere of Uranus. Ten moons were discovered by *Voyager 2*. The close-ups of the Uranian moon Miranda showed the small satellite to be dramatically fractured by geophysical forces. Miranda was found to have one of the most geologically diverse landscapes seen in the solar system[3-7].

THE EXPERIMENTS ON *VOYAGER 2*

Voyager 2 carried onboard a bank of 11 scientific experiments[8] designed to investigate a variety of planetary phenomena. Ten instruments are mounted on various locations of the spacecraft. The radio, used for communications with Earth, was also employed as a scientific instrument to probe planetary and satellite atmospheres (figure 2). The steerable scan platform of the spacecraft houses four instruments: the imaging science subsystem, infrared interferometer-spectrometer-and-radiometer, photopolarimeter and ultraviolet spectrometer.

Imaging science experiment

The experiment consists of two television cameras each with eight filters. The wide-angle camera has a



1. Cosmic ray detector
2. Plasma sensor
3. Wide angle imaging camera
4. Narrow angle imaging camera
5. UV spectrometer
6. IR spectrometer and radiometer
7. Photopolarimeter
8. Low energy charged particle detector
9. Antenna (3.7 M. dia.)
10. Hydrazine thrusters (16)
11. Spacecraft bus housing electronics
12. Planetary radio astronomy and plasma wave antenna
13. Nuclear thermoelectric generator
14. Magnetometers

Figure 2. *Voyager 2* spacecraft, showing the location of different experimental subsystems.

focal length of 200 mm and images in the range 4000 to 6200 Å. The narrow-angle camera has a focal length of 1500 mm with an imaging range of 3200 to 6200 Å. This experiment will enable photographing visible characteristics of the Neptunian system, and conduct searches for new satellites and ring material.

IR interferometer-spectrometer-and-radiometer

This is a telescope-based system that measures radiation in two regions of the infrared spectrum, 2.5 to 50 μm and 0.3 to 2.0 μm. It can measure temperatures and determine certain types of elements or compounds present in the atmosphere or on a surface. As a radiometer, it can measure the total amount of reflected sunlight at UV, visible and IR frequencies.

Photopolarimeter

It consists of a (0.2 m) telescope fitted with filters and polarization analysers. It measures the way its targets reflect light and determines their properties, such as polarization. It covers eight wavelengths in the region between 235 μm and 750 μm. It is meant to study the surfaces of moons, tiny aerosol particles in an atmosphere, and ring particles. It can infer the texture and composition of a solid surface, the density, particle sizes and composition of a planetary ring, as well as particle sizes and composition of particle hazes. It is also usable for observing stellar occultations.

Radio science experiment

Here the two-way radio communications link with Earth is used to conduct scientific investigations. Precise measurements of the phase and amplitude of the radio signal reaching the Earth through the atmosphere of planet and satellites are analysed to detect minute variations. It can thus probe both planet and satellite atmospheres and ionosphere if present. Studies of solar corona and characterization of ring structures around the planet are also possible.

Ultraviolet spectrometer

It consists of a grating spectrometer sensitive to UV radiation in the range 500 to 1700 Å and uses the principle of atomic emission and absorption. It enables the study of the composition of the atmospheres of Neptune and Triton and can also be used for UV astronomy studies by observing stellar objects.

Cosmic ray experiment

It has seven independent solid-state-detector telescopes and together they cover the energy range 0.5 to 500 MeV. It can measure the energy spectrum of electrons and cosmic ray nuclei.

Low-energy charged particle detector

Consisting of two solid-state detectors mounted on a rotating platform, the experimental sensitivity ranges from 15 keV to about 160 MeV. The two subsystems, one a low-energy particle telescope and the other a low-energy magnetospheric particle analyser, are used to characterize the composition, energies and angular distribution of charged particles in interplanetary space and within planetary systems.

Magnetometer experiment

It has 4 magnetometers to detect and measure magnetic fields. Two are low-field instruments mounted on a 10 m boom away from the field of the spacecraft, while the other two are high-field instruments mounted on the body of the spacecraft. The low-field magnetometers can detect fields as weak as 1/10000 of the strength of Earth's magnetic field while the high-field instruments are designed to detect fields stronger than 20 times Earth's magnetic field. They are used to study the interaction of the magnetic field with moons orbiting within it and observe the interplanetary and interstellar magnetic fields in the vicinity of the planet.

Planetary radio astronomy experiment

It uses two 10 m electric antennas as detectors. This is shared with the plasma wave subsystem. The range covered is 20 kHz to 40.5 MHz in the radio frequency band. The experiment will search and characterize a variety of radio signals emitted by Neptune and determine the relationship of these emissions to the moons, the magnetic field, atmospheric lightning and plasma environment. Planetary and solar radio bursts will be measured from different directions in space and correlated with measurements made from Earth.

Plasma wave subsystem

This experiment is designed to measure the electric field component of local plasma waves. It uses the two extendable ion electric antennas, which stretch at right angles to one another forming a 'V', and covers the range 10 Hz to 56 kHz. It is used to measure the density and distribution of plasma, interactions of plasma waves with energetic particles and the interactions of moons and ring arcs with the planet's magnetosphere.

Plasma subsystem

This experiment consists of 2 plasma detectors sensitive to solar and planetary plasmas—both positive ions and electrons with energies between 10 and 6000 eV. One detector points in the direction of Earth and the other points at right angles to the first. It is used to study the extent and configuration of the magnetic field and the nature and sources of internal plasma and also to observe the solar wind and its interaction with the Neptune system.

VOYAGER 2 SPACECRAFT

The *Voyager 2* spacecraft (figure 2), with a launch mass of 825 kg, is nuclear-powered, consists of about 5 million electronic parts, and uses onboard computer fault detection and response to protect itself. The spacecraft structure is called a 'bus', which resembles a large ten-sided box with each side containing a compartment to house the scientific instruments. The spacecraft is usually aligned so that its roll axis (and thus the high-gain antenna) points to Earth.

The 3.66 m high-gain antenna transmits data to Earth on two frequency channels. The X-band at about 8.4 GHz contains the science and engineering data with downlink data rates ranging from 4.8 to 21.6 kbps. The other channel in the S-band around 2.3 GHz transmits only engineering data on the state of health of the spacecraft at low rates of 40 bps.

Spacecraft stabilization and spacecraft manoeuvering are controlled by an onboard computer called the attitude and articulation control system (AACS), which also controls scan platform motion. *Voyager*'s attitude is maintained by gyro control and celestial control using Sun sensor and Canopus star tracker. Gyro control is used for special purposes and short periods of time, up to several hours. Four of the science instruments which need to be pointed at a target body are on the scan platform. The scan platform has motors and gears (called actuators) which slew the platform to point in various directions.

Spacecraft electrical power (~400 W) is supplied from three radioisotope thermoelectric generators which employ plutonium 238 to generate heat, which in turn is converted to electrical energy.

On occasions when the antenna is not pointing at Earth or when the spacecraft is being manoeuvred or when the spacecraft is behind the planet as seen from Earth, the data are stored in a digital tape recorder.

The central controller of the spacecraft (CCS) consists of two identical computer processors, their software, algorithms and some associated electronic hardware. The main functions of the CCS are to carry out instructions from ground to operate the spacecraft, gather science data, and to be alert for and respond to any problem with any of the spacecraft subsystems.

THE ENCOUNTER WITH NEPTUNE

Even though Neptune is 4.5 billion kilometres from Earth, the actual trajectory, the Grand Tour route of *Voyager 2*, that finally took the spacecraft to the vicinity of Neptune, involved traversal of nearly 7 billion kilometres of interplanetary space. The trajectory for approach towards Neptune (figure 3) was tailored in such a way that *Voyager 2* had a modest velocity component in the same direction as Neptune's motion around the Sun. This velocity component of the spacecraft was trimmed to be nearly the same magnitude as Neptune's orbital speed, i.e. 468,000 km/day. These matched speed kept both Neptune and *Voyager 2* from passing each other during most of the approach. In fact, the relative positions of the two were such that *Voyager 2* tended to stay close to the Sun–Neptune line which gave its cameras and other sensors a nearly 'full moon' view of anything in the Neptune system—the planet, Triton, Nereid, and other possible moons yet to be discovered. These high illumination lighting conditions gave the brightest view of the objects in the Neptune system and thus optimized the chances of discovering new objects in the system. The other velocity component, along the Sun–Neptune line, was over three times as fast as that along the orbital motion of Neptune and brought *Voyager 2* closer to Neptune at the rate of 1.45 million km per day.

The next important step was the selection of the aiming point, i.e. where exactly in space and time *Voyager 2* would come closest to Neptune. The selection[2] of the aiming point was dictated on one side by a variety of science objectives and on the other by the need to avoid endangering the vulnerable spacecraft to hazards of the Neptunian system environment, such as ring-arcs, atmosphere and radiation belts. The science objectives included

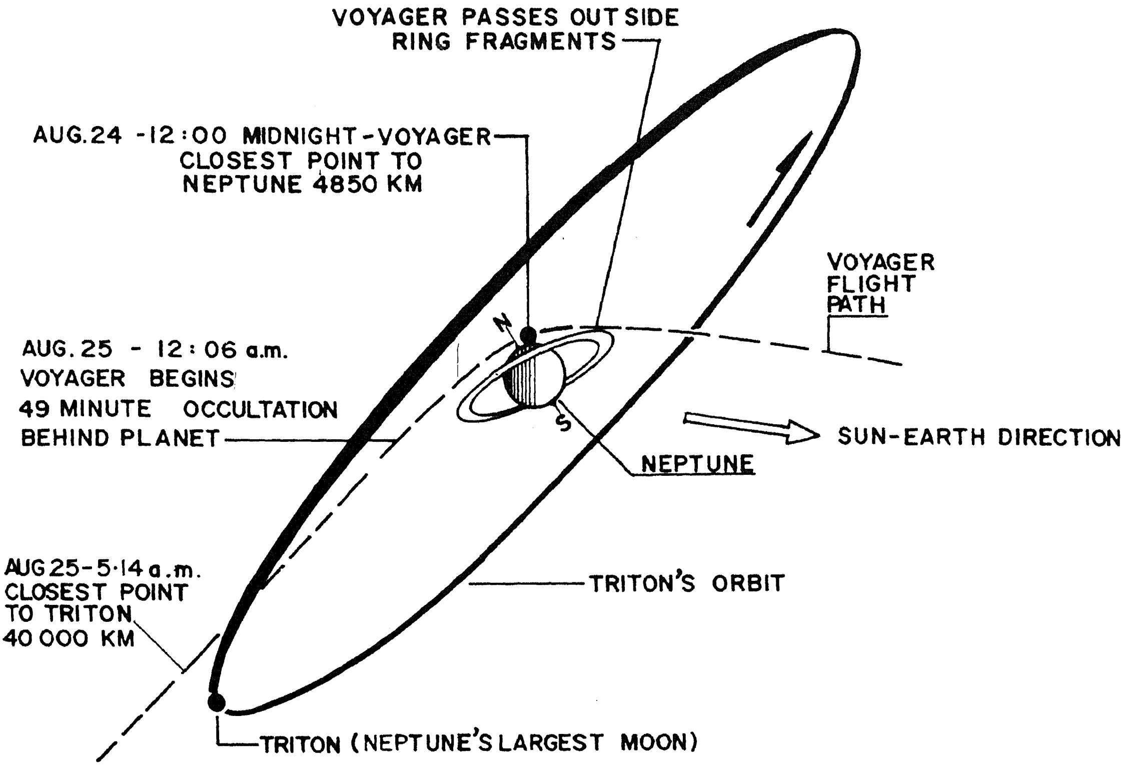

VOYAGER PASSES OUTSIDE RING FRAGMENTS
AUG.24 -12:00 MIDNIGHT-VOYAGER CLOSEST POINT TO NEPTUNE 4850 KM
VOYAGER FLIGHT PATH
N
AUG.25 - 12:06 a.m. VOYAGER BEGINS 49 MINUTE OCCULTATION BEHIND PLANET
S
SUN-EARTH DIRECTION
NEPTUNE
AUG 25-5·14 a.m. CLOSEST POINT TO TRITON 40 000 KM
TRITON'S ORBIT
TRITON (NEPTUNE'S LARGEST MOON)

Figure 3. Geometry of the Neptune and Triton encounter, with time in EDT.

a close look at Triton and a detailed study of Neptune, penetrating into its magnetosphere as well as passing behind the planet to ensure that the spacecraft sensors and the deep space network (DSN) antennas on Earth could take advantage of the high-value Earth and Sun occultation conditions such a trajectory could produce. Occultation by Triton was another interesting possibility to be kept in mind. Multiple views of the purported ring-arc system were also considered desirable. Selection of the aiming point was dictated by these considerations and set up the geometry and timing for all encounter events, thus controlling most of the science-related parameters. The aiming point selection for Neptune was one of the most challenging of all the *Voyager* encounters, including those for *Voyager 1*. The various considerations led to the selection of the northern polar region near the 'inner Triton locus' as the most desirable place by which to make a fly-past of Neptune. This required a close pass over the northern polar region of Neptune to gain sufficient gravitational deflection for a close encounter with Triton five hours later (see figure 2). Refinement of these calculations, taking due account of the environmental hazards posed by the ring-arc system, enabled fixing the exact aiming point. The selected aiming point enabled *Voyager 2* to skim Neptune's northern polar region 4850 km from the cloud-tops (at 04:00 UT on August 25) and make the closest approach to Triton at 40,000 km. The actual point of closest approach was only about 30 km away from the predicted position and the spacecraft arrived at this point only 4 min earlier than the time predicted a decade ago! The early arrival was no reflection on *Voyager's* performance, but rather on astronomy's uncertain knowledge of Neptune's exact position in the solar system. Since its orbital period is 165 years and since it was discovered only 144 years ago, astronomers have not earlier observed Neptune at this point in its orbit.

SCIENCE HIGHLIGHTS

The atmosphere

The weather systems on Neptune imaged by

Voyager 2 were truly remarkable. With only ~5% as much heat to generate winds as on Jupiter (including the internal heat source and the small energy absorbed from the Sun), the surprisingly dynamic atmosphere of Neptune, with its storms and strong retrograde winds of over 1100 km/h, will require for its understanding complex mathematical modelling and detailed knowledge of the various physical processes responsible for the phenomena. A new feature observed as the spacecraft approached Neptune was the Great Dark Spot, located about 20°–22° south latitude, with dimensions as large as Earth's. It is reminiscent of the Great Red Spot on Jupiter, also located ~20°–22° south latitude. Both appear to be resulting from large anticyclonic storms in their atmospheres. The ratios of the sizes of the Great Spots to those of the respective parent planets are strikingly equal. *Voyager 2* also observed high cirrus clouds that change rapidly and cast shadows on a deeper cloud layer some 50–75 km below.

Rings

Prior to the *Voyager* mission, ground-based observations of stellar occultations by Neptune had already given evidence for the existence of three narrow ring-arcs around the planet. Several models had been advanced to explain the ring-arcs. One of them required shepherding moons with orbits inclined to the plane of the ring-arcs. *Voyager 2* discovered that the ring-arcs are nothing but parts of complete but tenuous rings. *Voyager 2* in fact discovered three complete rings and a broad sheet of ring material. Shepherding moons which constrain the ring boundaries were however not detected. The non-uniform distribution in azimuth of the ring materials still remains a puzzle to be solved.

Magnetic field

The magnetometers on *Voyager* detected a modest magnetic field on Neptune, ~0.4 gauss, not much stronger than Earth's field. But the surprising thing was that the axis of the dipole field was tilted about 50° with respect to the rotation axis of the planet and the effective dipole centre was found to have a large offset from the centre of the planet. Similar tilt was also observed on Uranus, but then it was thought that since Uranus itself was tipped on its side, such a tilt need not be considered too surprising. Now, Neptune is certainly not tipped like Uranus, and the tilt of its magnetic axis is therefore due to other causes, including the location of the dynamo-current system, which produce the field, perhaps being closer to the surface of the planet. The trapped radiation belt of particles, although weak, powers the aurora observed on Neptune's largest moon Triton.

Moons

Prior to the *Voyager* mission, there were only two moons known to be associated with Neptune, viz. Triton and Nereid. *Voyager 2* discovered six new moons, all of them dark, all of them orbiting prograde and, surprisingly, even the largest of them—1989 N1, with a radius of ~200 km slightly larger than Nereid, whose radius is only 170 km—irregularly shaped and showing little geologic modification. Moon 1989 N1 escaped detection with ground-based telescopes because of its proximity to the relatively brighter Neptune.

Triton

Triton, the largest moon of Neptune, is roughly the size of Earth's Moon. Its radius is about 1360 km. It is the only large moon in the solar system with a retrograde orbit. Its average distance from Neptune is nearly the same as the Earth–Moon distance. The orbital and rotational periods of Triton are the same, viz. 5.88 days, which implies that Triton shows the same face to Neptune, as does our Moon to Earth. *Voyager 2* discovered a thin atmosphere of methane and nitrogen on Triton. The surface pressure is only about 10 microbars. *Voyager* images also revealed the mottled appearance of the surface of Triton, with a variety of geological features, including possibly liquid nitrogen-spewing volcanoes that were active in the recent past. Triton also revealed an aurora, which implies the presence of a magnetic field and possibly a liquid core. With a surface temperature of only 37 K, Triton is so far the coldest object seen up close in the solar system.

TOWARDS MORE DISTANT ENCOUNTERS

Scarred by the violent radiation of Jupiter and the dust belt of Neptune and with its trajectory swung south of the ecliptic plane at an angle of 48° after the Neptune–Triton encounter, *Voyager 2* is heading on a new mission—a hunt for the edge of the Sun's influence and the shores of the great stellar ocean. Speeding away from the solar system at a rate of 60,000 km/h, and with the nuclear batteries capable of ensuring the operation of *Voyager 2* at least for another 25 years, the spacecraft could continue to explore the regions of space as far as 130 AU from the Sun. Scientists believe that it is travelling towards the leading edge of the magnetic bubble which represents the farthest reach of the Sun's magnetic field. A stream of protons, electrons and, to a lesser extent, helium nuclei, called solar wind, flows steadily outward from the Sun at velocities of the order of 400 km/sec. The region where the supersonic solar wind suddenly goes subsonic and where the influence of the Sun's magnetic fields ends is called heliopause. No one knows exactly where the solar system ends and interstellar space begins. Nevertheless, the heliospheric boundary is believed to be somewhere between 50 and 150 AU from the Sun. Mission planners estimate that *Voyager 2* will probably cross the heliopause around the year 2012 and enter true interstellar space. The first direct measurements of the environment outside our solar system, including interstellar magnetic fields and charged particles, will be hopefully possible at this time.

Beyond the heliopause, riding on its momentum, *Voyager 2* will continue its silent journey through the Milky Way, eventually passing within 4.03 light years of Bernard's star in the year 8571 and by Proxima Centauri in another 11,500 years. In the year 26262, *Voyager 2* should enter the Oort cloud, a region of comets orbiting the Sun. Travelling further, *Voyager 2* should pass within 4.3 light years of Sirius, the 'Dog star', in the year 296036. By that time, the wandering spacecraft will be 14.64 light years from home! According to the *Voyager* navigator, Tony Taylor, 'There is something wonderful about having a piece of humanity wandering among the stars; it is like having immortal children.'

Voyager 2, by sending down breathtaking views of other worlds, has created a growing awareness in us of Earth as a planet, and a very fragile one. The legacy of *Voyager 2* can be best expressed through the words of the poet T. S. Eliot: 'We shall not cease from exploration and the end of all our exploring will be to arrive where we started and know the place for the first time.'

ACKNOWLEDGEMENTS

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CURRENT SCIENCE—50 YEARS AGO

THE INDIAN CHEMICAL INDUSTRY*

THE ten weeks since the declaration of the War, have been witnessing a steady inflation in the price of drugs and chemicals in this country. The manufacturing industries whose maintenance is largely dependent upon the import of foreign accessories and raw materials, have already begun to feel the pinch of a diminishing supply of their essential needs. The Conference of Chemical Manufacturers held recently at Calcutta, under the auspices of the Indian Chemical Manufacturers' Association has focussed the attention of the public on the imminent shortage of drugs and chemicals in India. The country has now become painfully conscious of its large dependence upon foreign sources for its supply of the principal drugs and chemicals.

The country was confronted with a similar crisis twenty-five years ago, during the Great War of 1914–18. Since then, the Indian Chemical Industry has made some progress; the pharmaceutical industry, for instance, has been trying to establish itself and has survived keen foreign competition; small nuclei of heavy chemical industry which now exist in the country, are manufacturing sulphuric acid; one or two works for the manufacture of alkali, are also under active consideration. But these could satisfy only a small fraction of the growing needs of the country. At the moment, India consumes, annually, about five crores rupees worth of drugs and chemicals, of which at least seventy-five per cent is imported from abroad.

The Chemical Industries in India group themselves into three main classes:—(1) Those which are in the process of establishing themselves on a fairly firm foundation, (2) those which are yet in their infancy and which, on that account, require careful protection and consolidation and (3) those which await exploitation. The pharmaceutical industry which falls under the first group, is in immediate need of rapid expansion to satisfy the increased demand caused by the total stoppage or partial curtailment of imports. The development of this industry, which is urgent, could have been easily secured, if we were not obliged to depend upon foreign sources even for some of the basic raw materials. The textile industry is faced with a similar situation because of the rapidly diminishing supplies of wetting, bleaching and dyeing materials.

Many of the industrial enterprises now flourishing in the country can continue to function only if an unrestricted supply of the imported raw materials and spares of manufacturing machinery is ensured. It would be difficult to point out one single chemical industry in this country which can be considered self-sufficient and which will not be more or less crippled for want of foreign supplies. This unhappy state of affairs is the natural consequence of the policy of wholesale transplantation of industries which the country has been adopting in the past. There have been several instances in which even erecting engineers and technical experts have been imported to run the industry. The adoption of such a policy was no doubt necessary at a time when the industries were still undeveloped. Very few of the industries can claim to have originated through our own creative effort, which has yet to play its part in the future development of industries.

It may be said that the country is now on the threshold of the second stage when the creative faculty, latent in the country, should be stimulated and mobilised for the promotion of industrial advancement. This is not going to be an easy task for a country which has so far lacked competent, disinterested and broad-minded leadership in this field of national economy.

The next phase of industrial development in this country should consist in the consolidation of the existing industries; they should be rendered less and less dependent upon foreign imports. Subsidiary industries helping to produce accessories and intermediates should be organised. The possibility of utilising substitutes of Indian origin should be intensively and systematically explored. In the domain of pharmaceuticals, for example, it would be easy to find substitutes, perhaps more efficacious, but the industry is yoked under a highly conservative British Pharmacopoeia. It is for the *Indian Medical Research Council* to take up this matter and examine the situation.

The designing and fabrication of chemical plant and machinery leading to the establishment of

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chemical engineering industries in the country, should be immediately taken up for serious consideration. The country possesses the necessary equipment and talent; we have large foundries and machine shops and skilled and capable workmanship is available. We may be lacking in some of the specialised materials of construction like stainless steels and special alloys, but we could, for the moment, do without them. It is a matter of profound regret that the Central Government could not see their way to subsidise Sir M. Visvesvaraya's scheme for founding an automobile industry in this country, as this would have facilitated the establishment of the chemical engineering industries by providing the necessary background. The present opportunity should not be lost in laying the foundations of this industry on sound lines.

The Government of India have suggested that Indian Manufacturers might prepare a consolidated statement of their requirements of heavy chemicals and obtain them through Messrs. Imperial Chemical Industries, who are in close touch with the world markets with regard to these commodities. While this arrangement will provide a temporary and easy solution to the problem, this remedy will, in the long run, prove worse than the disease, as it will only serve to perpetuate our dependence on foreign supplies to nourish our industries. The Central Government should take a broader and more generous view of the problem and help the establishment of these key industries in the country. Such a step would be in the best interests not only of India, but also of the Empire. The industrial prosperity of Canada, Australia and other Dominions, has proved to be a source of great strength to Britain in the present crisis; flourishing chemical and engineering industries in India would, in a similar manner, constitute great assets to Britain in war as well as in peace.

It is for the Central and Provincial Governments to move in the matter of organising and mobilising the material resources and technical talent and help the country to enter on the second phase of industrial development. It would be a grievous mistake if the Government should lose this opportunity. A Board of Scientific and Industrial Research or a National Research Council should be constituted to deal with all the aspects of the problem. The Industrial Research Bureau which is now miserably staffed and financially starved, may be reconstituted into a bigger and more comprehensive body and provided with ample funds to finance schemes of industrial research.

ALCOHOL AS MOTOR FUEL*

Now that a beginning is being made in India in the use of alcohol for mixing with petrol as fuel in motor vehicles, trials conducted elsewhere on the suitability of such mixtures should be both valuable and interesting. The report of an elaborate trial with alcohol used nearly straight and in mixtures of varying proportions with petrol for driving a motor car appears in the *Philippine Agriculturist* 28, No. 2 (A. L. Teodoro, Fifty thousand kilometres on alcohol as motor fuel). The trials relate to two groups, one comprising the use of nearly straight alcohol (gasoline being only 3% and 5%) and the other comprising mixtures in which the gasoline was 10, 30, 50, 70, 80 and 90%. The car used in the trials was a De Soto De Luxe Sedan (1929 model) which had run on gasoline for four years during which a distance of 10,678 miles had been driven. Slight alterations were made to the car before the trials such as, enlarging the diameter of the high speed metering jet, and of the area of the pump discharge jet; ignition timing was set 5 to 14 degrees ahead of the usual adjustment for gasoline, and idling adjustment was changed according to the kind of alcohol motor fuel used. Details of the behaviour of the engine in respect of starting, acceleration, power, engine wear, corrosion and economy of operation are given in full. Likewise for each one of the fuels used particulars under working conditions and of the number of miles driven are also given, with full numerical data, for all of which reference to the full report is commended. As the result of these trials in which quite 50,000 kilometres were run it is concluded that the car performed very satisfactorily on these alcohol fuels for a period of five years. No difficulty was encountered in starting except when the engine carburetion and ignition systems were faulty and when the driver improperly used the choke. As much power as could be produced with

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gasoline was obtained with the alcohol fuels. No sticking of the piston valves was noted. The mileage increased as the amount of gasoline increased in the mixture; thus with the nearly straight alcohol fuels the mileage was only from 8.9 to 9.8 miles per gallon, while with the gasoline mixtures the mileage rose to 11.3, 12.7, 14.4, 15.3, 16 and 17.4 as the gasoline percentage rose from 10 to 30, 50, 70, 80 and 90% respectively. For the proper utilization of nearly straight alcohol proper adjustment of carburetion and ignition and care in manipulating the parts which control these systems are specially mentioned as requirements.

A. K. Y.

ANNOUNCEMENTS

Workshop on Recent Advances in Chromatographic Techniques

Place: Madras
Date: 14–20 December 1989
Contact: Dr B. Sivasankar
Centre for Biotechnology
Anna University
Madras 600 025

Workshop on Management of Research and Development

Place: Pune
Date: 20–24 January 1990
Contact: Programme Coordinator
Engineering Staff College of India
Visvesvaraya Bhavan, Khairatabad
Hyderabad 500 004

Workshop on Clinical Pharmacokinetics

Place: Lucknow
Date: 26–29 March 1990
Contact: Dr P. K. Grover
Pharmacokinetics and Metabolism Divn
Central Drug Research Institute
Lucknow 226 001

Ninth Annual Convention & Conference of Society for Information Science

Place: Goa
Date: 18–20 January 1990
Theme: Local Area Network (LAN): Its significance in information system
Contact: Dr P. C. Bose
Society for Information Science
PID Building
Hillside Road
New Delhi 110 012

A Six-Week Course on Wind Energy for Water Pumping

Place: Enschede, The Netherlands
Date: 5 February to 16 March 1990
Contact: Reading/CWD Wind Energy Course
C/o Consultancy Services Wind Energy
Developing Countries
P.O. Box 85
3800 AB Amersfoort
The Netherlands

ARTICLES

COMMENTS ON THE BENOFY AND QUAY THEORY OF THERMOMAGNETIC EFFECTS

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ABSTRACT

The claim by Benofy and Quay that Fourier's inequality ($J_q \cdot \nabla T \leq 0$) contains a further thermodynamic principle beyond that contained in the Second Law of Thermodynamics is investigated. It is shown that the inequality is not in contradiction with the usual assumptions of linear irreversible thermodynamics, and when properly interpreted, the inequality is not violated as is claimed. It is not necessary to postulate a 'change in material coefficients' for thermo-electrical phenomena.

INTRODUCTION

Benofy and Quay (henceforth abbreviated BQ)[1] claim that Fourier's inequality $J_q \cdot \nabla T \leq 0$, where J_q is the heat current vector and T the temperature, contains a further thermodynamical principle beyond that contained in the Second Law of Thermodynamics. BQ explicitly state that their treatment refers to conductive heat only in an early part of their paper[2], and in this respect there may well be (within the classical, non-statistical continuum scheme) some validity in their arguments. However, later in their paper there is a tendency to treat this form of heat as the only form of thermal energy flux.

It is (as will be shown) this failure to recognize other forms of thermal energy flow that has led BQ to misunderstand such works as Callen's Linear Irreversible Thermodynamics (LIT) formulation of magnetothermoelectric effects[3]. They seem to have misunderstood that while Callen's equations predict non-zero isothermal heat currents, such currents are due to 'heat of transport' and not conductive heat, and therefore do not violate Fourier's inequality, which refers to conduction alone. By 'heat of transport' is meant the thermal energy transported by a current of particles as discussed following equation (2) below and also in Callen's article[5]. This misunderstanding is evident in their objection to Callen's equations requiring that heat should flow in the absence of a temperature gradient due to the 'mutual interference of heat flow and electric current flow in a system'[3,5]. Furthermore, BQ seem to imply that the LIT 'coupling' of various forces and fluxes are the root of the problem regarding violations of the Fourier inequality.

"But, unlike previous workers in this area, we do not 'couple' the equations themselves by adding any term depending on electric field to Fourier's equation or one depending on thermal gradient to Ohm's Law. For, it is such 'couplings' that generate theoretical violations of Fourier's inequality. Rather, we add one further equation, long known in its rudimentary form, which 'couples' the two gradients in such a way as to give the full set of empirically observable relations while maintaining Fourier's Principle intact."

THE INTERPRETATION OF LIT AND THE CONNECTION TO THE BQ THEORY

It must be understood that in LIT heat processes involve both conduction by a 'medium' and transport by particle currents. In the case of the treatment of magnetothermoelectric effects by Callen and BQ, the medium is a flat, uniform (metallic) conductor. Taking the simplest possible case, consider the LIT expression for a one-dimensional flow of heat and an electric current[6,7] in an electrical conductor given by the equations:

$$\mathbf{J}_q^{\mathrm{tot}} = -L_{qq} \nabla T/T^2 - L_{qe} \nabla E/T, \qquad (1)$$

$$\mathbf{J}_e = -L_{ee} \nabla E/T - L_{eq} \nabla T/T^2, \qquad (2)$$

where we have neglected (as with Callen) the concentration of the electrons and only consider the

Fourier Heat conduction law; Linear irreversible thermo-
dynamics. Benofy and Quay

electrical potential term E, the temperature T and their respective gradients as components of the force. The L's are the temperature-dependent coupling coefficients. $\mathbf{J}_e$ represents the electrical current flow vector and $\mathbf{J}_q^{\text{tot}}$ the thermal energy flow vector, where we have written $\mathbf{J}_q^{\text{tot}}$ as a sum with contributions from the thermal (non-conductive thermal transport energy flow due to electrons, i.e. heat of transport) $\mathbf{J}_q^{\text{trans}}$ and a Fourier-like conductive heat flow term $\mathbf{J}_q^c$ defined respectively by the equations:

$$\mathbf{J}_q^{\text{trans}} = -L_{qe}\nabla E/T \tag{3}$$

and

$$\mathbf{J}_q^c = -L_{qq}\nabla T/T^2.$$

It can be shown[8] that

$$-L_{qe}\nabla E/T = \varepsilon T K_e \nabla E, \tag{4}$$

and in the absence of a thermal gradient, $\mathbf{J}_e = -K_e \nabla E$, so that

$$\mathbf{J}_q^{\text{tot}}|_{\nabla T=0} = \mathbf{J}_q^{\text{trans}} = \varepsilon T \mathbf{J}, \tag{5}$$

where εT is neither more nor less than the thermal energy per unit current (K_e is the electrical conductivity) per unit area.

Equations (1) and (2) are first order local equations. If there is conversion, i.e. dissipation where the electrical potential work is dissipated as heat to the lattice, then local heating would cause a near instantaneous temperature change in the lattice so that the resulting temperature gradient would cause heat to flow by a lattice conductive mechanism as given in (1). There is no reason why (1) would preclude the possibility of conductive heat and thermal energy of transport from moving in opposite directions.

With the assumption of 'local equilibrium' in LIT as a basic postulate[9], we can interpret T in (5) as the local temperature and $\varepsilon = \varepsilon(T)$ as a locally defined Seebeck coefficient which will of course depend not only on the temperature but also on the nature of the medium[10].

Subtracting the effects of Joulean heating (by, for instance immersing the conductor in a constant temperature bath) there can be no net conduction of heat along the direction of electron flow in the isothermal situation, in accordance with the Fourier principle. However, electrons will flow along the conductor and carry thermal energy in the direction of their flow. Since the system is characterized by a constant temperature, there will only be transfer of thermal energy by transport and no conduction.

The LIT formulation therefore does not contradict the ideas presented by BQ provided both refer to conductive heat, whereas the 'rational thermodynamics' school of Truesdale and others are not compatible with the BQ assertions regarding conductive heat[11].

It is only when BQ regard their $\mathbf{q}$ vector as constituting the total heat current that misunderstandings with the LIT theory become apparent, since one of the BQ assumptions is that[12] $\mathbf{q}=0$ entails $\nabla T=0$. Equation (1) does not support this view if the BQ $\mathbf{q}$ is identified with $\mathbf{J}_q^{\text{tot}}$. There is no other way in which it is possible for LIT to violate the Fourier inequality unless this identity is made. BQ have not shown why any violations should occur, although they insist that the LIT coupling coefficients cause violations to the Fourier inequality.

BQ introduced the idea of the 'change of material coefficients'[13] as an alternate way of viewing coupled phenomena[13] to circumvent the purported difficulties inherent in other approaches. Such a viewpoint may in part be traced to the influence of Campbell[14,15] in his experimental description of thermomagnetic effects in which the actual form of the kinetic equations does not change; the result of the interaction of a magnetic (and possibly other fields) simply serves to modify the kinetic coefficient pertinent to the flow. For instance, if the resistance R_e is said to be defined for a conductor according to the phenomenological equation:

$$\mathbf{J}_e = (1/R_e)\Delta E, \tag{6}$$

where ΔE is a fixed potential difference across the uniform conductor for current flow $\mathbf{J}_e$ (see reference 16 for BQ interpretation of magneto-resistance), for large enough fixed ΔE (i.e. a voltage source across the conductor) the current through the conductor will vary as the applied magnetic field (or other field) gradient varies due to the presence of the Lorentz and lattice forces on the moving electrons due to the magnetic field gradient; what has occurred is that a seat of e.m.f. has been created within the conductor which modifies the flow of electrons, so that from (6), Campbell concludes that a 'change' of resistance has occurred. If there exists an active Seebeck field in the conductor due to a temperature gradient instead of a magnetic filed, a similar situation as for the magnetic case would ensue, i.e. the current will vary (for fixed ΔE) as the temperature gradient varies.

However, it may be argued that definition (6) only applies to isothermal conductors with no other interactions apart from the external field ΔE. If an active e.m.f. seat is induced within the conductor (e.g. by say the Seebeck field due to a temperature gradient) then it would tend to oppose or assist the field due to an external seat of e.m.f. Hence a different steady state current would flow through the conductor for the same voltage difference ΔE if these fields were present compared to the case when they are not. The 'actual' resistance of the material does not in fact change if we properly account for the induced e.m.f.

We now give an example which will also provide the proper coupling relationship between the electric field and temperature field, other than the one provided by BQ[17], which cannot be correct, as will be shown.

For the Seebeck effect, for example, the non-uniform temperature distribution will complicate the measurement of resistance because of the induced e.m.f. This can be allowed for, however, by defining the passive resistance of a conductor with a non-uniform temperature distribution maintained at T_1 and T_2 at the ends by

$$R_e = \overline{R(T_1)} + \int_{T_1}^{T_2} R(T)\cdot l'\,\mathrm{d}T \qquad (7)$$

with $l' = \partial l/\partial T$. Here, $R(T)$ is the resistance per unit length, and $\overline{R(T_1)}$ the resistance of the conductor at temperature T_1 and l the length parameter of the conductor. The change of resistance may be determined by isothermal measurements of the resistance at different temperatures.

The heat dissipated in an isothermal passive resistor is given by the total work done by the field and is given per unit length by the term $-\mathrm{d}E/\mathrm{d}x\cdot J_e$ which may be termed the isothermal Joulean dissipation. For a resistance of unit length, we may write:

$$\text{Joulean dissipation} = \mathbf{J}_e^2 R_e = -\mathrm{d}E/\mathrm{d}x\cdot\mathbf{J}_e. \qquad (8)$$

On the other hand, the total heat dissipated in unit length of a conductor[18] in the steady state in the presence of both an electrical and thermal gradient may be written as (neglecting the Fourier conduction component):

Total heat dissipated =

$$-T\mathbf{J}_e\cdot\frac{\mathrm{d}\varepsilon}{\mathrm{d}x} + \mathbf{J}_e^2/K_e = -T\mathbf{J}_e\cdot\frac{\mathrm{d}\varepsilon}{\mathrm{d}x} - \mathbf{J}_e\cdot\frac{\mathrm{d}E}{\mathrm{d}x} - E\frac{\mathrm{d}T}{\mathrm{d}x}\cdot\mathbf{J}_e \qquad (9)$$

where

$$\mathbf{J}_e^2/K_e = \mathbf{J}_e^2 R_e = -\mathbf{J}_e\cdot\mathrm{d}E/\mathrm{d}x - \varepsilon\cdot\mathrm{d}T/\mathrm{d}x\cdot\mathbf{J}_e. \qquad (10)$$

Thus, in the presence of a thermal gradient, the Joulean dissipation $\mathbf{J}_e^2 R_e$ is composed of two terms, the isothermal Joulean dissipation $-\mathbf{J}_e\cdot\mathrm{d}E/\mathrm{d}x$ and a correction term linear in the temperature gradient which may be called the 'Seebeck' heat $-\varepsilon\cdot\mathrm{d}T/\mathrm{d}x\cdot\mathbf{J}_e$.

We may simulate a unit length of the conductor by the circuit element depicted in figure 1 where R_e is the intrinsic resistance, V_1 the Seebeck active e.m.f., and $\mathrm{d}E/\mathrm{d}x$ is the actual e.m.f. across the segment whose surfaces are at a and b.

Then Kirchoff's voltage law gives for the above system:

$$\mathrm{d}E/\mathrm{d}x = -\mathbf{J}_e R_e + V_1, \qquad (11)$$

where V_1 is $-\varepsilon\ \mathrm{d}T/\mathrm{d}x$, the Seebeck active e.m.f. field. Thus from (11)

$$\mathbf{J}_e^2 R_e = \mathbf{J}_e^2/K_e = -\mathrm{d}E/\mathrm{d}x\cdot\mathbf{J}_e - \varepsilon\,\mathrm{d}T/\mathrm{d}x\,\mathbf{J}_e \qquad (12)$$

as before in (10).

If $\mathbf{J}_e \to 0$, (i.e. the Joulean heat is made to vanish) then the actual work done $-\mathrm{d}E/\mathrm{d}x\cdot\mathbf{J}_e$ in a unit length is numerically equal to the Seebeck heat loss[14]. The terms ϕ and $\nabla\phi$ used by BQ[1] are equivalent to our E and $\mathrm{d}E/\mathrm{d}x$ (in one dimension).

When $\mathbf{J}_e^2 R_e \to 0$, then (12) yields

$$\mathrm{d}E/\mathrm{d}x|_{\mathbf{J}_e=0} = -\varepsilon\nabla T. \qquad (13)$$

This relation defines the Seebeck coefficient ε (ref. 20). BQ write instead

$$\nabla\phi = \tau\nabla T, \qquad (14)$$

where τ is the Thomson coefficient.

Equation (14) is supposed to represent the link between the electrical potential and temperature

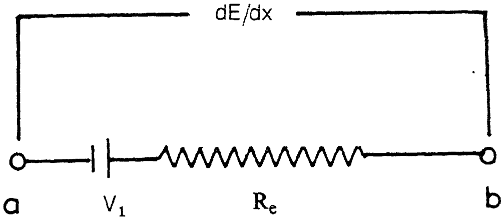

Figure 1.

gradient, "long known in its rudimentary form"[21]. The only reference to any such relationship is in one of Bridgman's theoretical constructs. If they were influenced by this construct, then it appears that they have misconstrued Bridgman's expression[22]

$$(\text{e.m.f.})\, w = \sigma(\mathrm{d}T/\mathrm{d}x), \tag{15}$$

where σ is the Thomson coefficient in Bridgman's notation. The BQ e.m.f. $\nabla\phi$ refers not to the 'conventional' potential difference but rather to Bridgman's hypothetical 'working' e.m.f. denoted (e.m.f.) w which is not a directly observable quantity but is defined in terms of the rate at which heat is absorbed when current flows[23].

In any case, it is not correct to use (14) as a link between the electrical potential and the temperature gradient.

CONCLUSIONS

It is conceivable that the 'change in material coefficients' may feature in a higher order expansion of flux expressions than is used in LIT (which is a first order expansion) but it would not be appropriate to equate these changes with the first order approximations characteristic of such theories as Onsager's LIT. BQ, however, neglected the importance of thermal transport and convective heat, and required the "change in material coefficients" as a device to compensate for this neglect. However, since conductive heat transfer does not involve the same mechanism as the other forms of thermal exchange, it follows that the BQ theory is incomplete if not flawed. We have shown that LIT is a consistent theory to first order and yields the correct convergence in the restricted treatment of thermo-electric effects above. The BQ hypothesis regarding the Fourier inequality for the conductive part of the heat current still remains a very interesting and original conjecture.

It must be stressed that the above derivations are based on 'per unit length', of material. It will be shown in future communications that a more detailed analysis of the above implies further generalizations of the Kelvin relations for thermocouples and that Onsager-like reciprocity may be derived from the conjecture on Fourier heat conduction within the LIT framework which couples forces and fluxes linearly with no reference whatsoever to the Onsager hypothesis of detailed balance, microscopic reversibility and the regression of fluctuations. Finally, a certain degree of scepticism is warranted in regard to the Bridgman equation and its experimental verifiability.

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VARIATION OF DIRECTIVITY AND QUALITY FACTORS OF CIRCULAR PATCH MICROSTRIP ANTENNA IN PLASMA

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ABSTRACT

Using linearized hydrodynamic theory the radiation properties of a circular patch microstrip antenna in the plasma media are studied. Directivity and quality factors for different plasma-to-source frequencies are calculated and plotted.

INTRODUCTION

MICROSTRIP antennas are replacing old and bulky antennas in modern rockets and satellites[1-4] because of their light weight and better aerodynamic properties. An antenna mounted on space vehicle encounters plasma media and generates electro-acoustic waves in addition to electromagnetic waves. Bhatnagar and Gupta[5] studied the radiation properties of a circular patch antenna in a multicomponent plasma media.

Two widely used mathematical theories describing the plasma state involve the use of hydrodynamic equations and the Boltzmann equations in conjunction with Maxwell's equations. The use of Boltzmann equation requires many assumptions. In this study we use hydrodynamic equations to describe the properties of plasma. The assumptions regarding the state of plasma and the basic equations governing radiations have been mentioned elsewhere[6, 7].

RADIATED POWER AND DIRECTIVITY

The circular patch microstrip antenna is shown in figure 1. The fields inside the cavity are obtained by[4]

$$E_z = k_1^2 J_n(k_1 \rho) \cos n\phi. \tag{1}$$

For dominant reflection action at the circular dielectric to plasma boundary at $\rho = a$, the tangential field $H_\phi = j\omega\epsilon_r J_n'(k_1\rho)\cos n\phi$ is very small and the values are given by

$$J_n'(k_1 a) = 0. \tag{2}$$

Following the method adopted by Bhatnagar and Gupta[8] and equations for EM and P mode fields[5], the radiated power is given by

EM mode:

$$P_e = \frac{(hk_1^2 a\beta_e J_n(k_1 a))^2}{1920} I_1, \tag{3}$$

here

$$I_1 = \int_0^\pi [\{J_{n+1}(\beta_e a \sin\theta) - J_{n-1}(\beta_e a \sin\theta)\}^2 + \cos^2\theta.$$

$$\{J_{n+1}(\beta_e a \sin\theta) + J_{n-1}(\beta_e a \sin\theta)\}^2] \times \sin\theta \, d\theta. \tag{4}$$

β_e is the propagation constant in the EM mode, h the thickness of substrate and a the radius of the patch.

P mode:

$$P_p = \int_0^{2\pi}\int_0^{\pi}\left[\sum_{j=1,2} \frac{K_j J_n^2(\beta_{pj} a \sin\theta)\sin(\beta_{pj} h\cos\theta)}{\sin(\beta_{pj} h \cos\theta)}\right]^2$$

$$\sin^2\phi \cdot \sin\theta \, d\theta \cdot d\phi \tag{5}$$

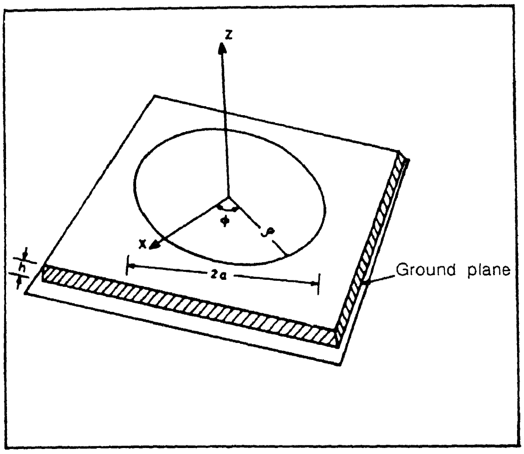

Figure 1. Geometry of circular patch microstrip antenna.

here

$$K_j = \frac{(1-\alpha_j)^2 \omega_{pe}^2 k_1^4 h^2 J_n^2(k_1 a)}{16 \beta_{pj} \varepsilon_0 (1+\tau\alpha_j^2)\omega u_e^2},$$

$$k_1 = \omega(\mu_0 \varepsilon_0 \varepsilon_r)^{\frac{1}{2}} \quad \text{and} \quad \tau = m_i u_i^2 / m_e u_e^2$$

α_1 and α_2 are the roots of the equation

$$T_{21}\alpha_j^2 + (T_{11} - T_{22})\alpha_j - T_{12} = 0, \tag{6}$$

$$\left.\begin{aligned} T_{11} &= \frac{\omega^2}{u_e^2}\left[1 - \frac{\omega_{pe}^2}{\omega^2}\right], & T_{12} &= \omega_{pi}^2/u_i^2 \\ T_{21} &= \omega_{pe}^2/u_e^2, & T_{22} &= \frac{\omega^2}{u_i^2}\left[1 - \frac{\omega_{pi}^2}{\omega^2}\right]. \end{aligned}\right\} \tag{7}$$

The wave number β_{pj} is given by the roots of the equation

$$\beta_{pj}^4 - \beta_{pj}^2(T_{11} + T_{22}) + (T_{11}T_{22} - T_{12}T_{21}) = 0 \tag{8}$$

and is related to α_j by

$$\beta_{pj}^2 = T_{11} + T_{21}\alpha_j, \tag{9}$$

ω_{pe} is the angular plasma frequency of electron, ω the angular frequency, u_e and u_i are r.m.s. thermal velocities of electron and ion respectively.

The directivity of an antenna is defined as the ratio of maximum power density to average power density. From the previously calculated far fields[5] and the total radiated power, the directivity of circular patch microstrip antenna in plasma excited by $n=1$ mode in the

EM mode:

$$\begin{aligned} D_e &= (\beta_e a)/120\, G_e \\ &= 8/\int_0^\pi [\{J_{n+1}(\beta_e a \sin\theta) - J_{n-1}(\beta_e a \sin\theta)\}^2 + \\ &\quad + \cos^2\theta\{J_{n-1}(\beta_e a \sin\theta) + \\ &\quad + J_{n+1}(\beta_e a \sin\theta)\}^2] \sin\theta \, d\theta. \end{aligned} \tag{10}$$

P mode:

$$D_p = \frac{4\pi \sum_{j=1,2} \left\{\dfrac{\sin(\beta_{pj} h \cos\theta)}{(\beta_{pj} h \cos\theta)}\right\}^2 K_j \sin\phi \, J_n^2(\beta_{pj} a \sin\theta)}{\sum_{j=1,2} \pi K_j \displaystyle\int_0^\pi \frac{\sin(\beta_{pj} h \cos\theta)}{(\beta_{pj} h \cos\theta)} J_n^2(\beta_{pj} a \sin\theta) \cdot \sin\theta \, d\theta}. \tag{11}$$

The values of D_e and D_p are calculated and plotted for different values of ω_{pe}/ω (figure 2).

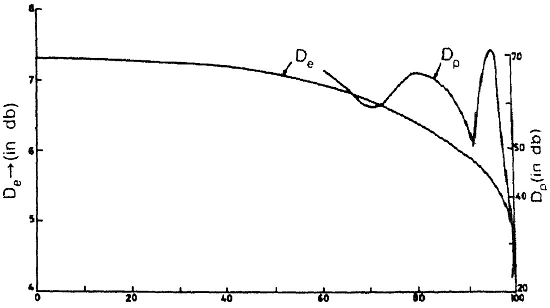

Figure 2. Variation of directivities (D_e and D_p) with plasma frequency.

QUALITY FACTOR

A parameter specifying the frequency selectivity of a resonant circuit is the quality factor. It is defined as the ratio between the energy stored in the system and the energy lost. At resonance, the energy stored can be calculated from either the maximum magnetic field or from the maximum electric field. In the present case, the energy stored is

$$\begin{aligned} W_t &= \tfrac{1}{2}\varepsilon |E|^2 \, dV \\ &= \frac{h k_1^4 J_n^2(k_1 a)}{8\omega f \mu}\{(k_1 a)^2 - n^2\}, \end{aligned} \tag{12}$$

and

$$Q_{\text{rad}} = \omega W_t / P_L. \tag{13}$$

The total power lost P_L, includes the power radiated and the power attenuated. The power attenuated is

$$\begin{aligned} P_A = &\left\{\frac{\pi f \mu}{\sigma}\right\}^{\frac{1}{2}} k_1^4 \frac{J_n^2(k_1 a)}{2(\omega\mu)^2} \cdot \{(k_1 a)^2 - n^2\} + \\ &+ \frac{h \tan\delta \, k_1^4 J_n^2(k_1 a)}{8\mu f}\{(k_1 a)^2 - n^2\}. \end{aligned} \tag{14}$$

The quality factor becomes for the

EM mode:

$$Q_e = \left[\frac{1}{h(\pi f \mu \sigma)^{\frac{1}{2}}} + \tan\delta + \frac{h f \mu (\beta_e a)^2 I_1}{240\{(k_1 a)^2 - n^2\}}\right]^{-1}. \tag{15}$$

P mode:

$$Q_p = \left[\frac{1}{h(\pi f \mu \sigma)^{\frac{1}{2}}} + \tan\delta + \frac{\mu f \omega_{pe}^2 h L}{2\varepsilon_0 \omega u_e^2\{(k_1 a)^2 - n^2\}}\right]^{-1}, \tag{16}$$

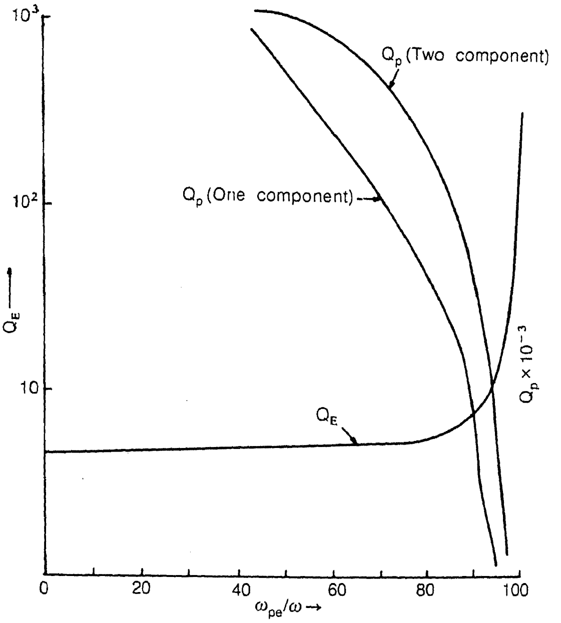

Figure 3. Variation of quality factor (Q_e and Q_p) with plasma frequency.

here

$$L=\int_0^{2\pi}\int_0^{\pi}\left\{\sum_{j=1,2}\frac{(1-\alpha_j)^2 J_n^2(\beta_{pj}a\sin\theta)\sin(\beta_{pj}h\cos\theta)}{\beta_{pj}(1+\tau\alpha_j^2)\beta_{pj}h\cos\theta}\right\}^2$$

$$\sin^2\phi\sin\theta\,\mathrm{d}\theta\,\mathrm{d}\phi, \qquad (17)$$

σ is the conductivity of the metallization, μ the permeability of the medium, $\tan\delta$ the loss tangent of the dielectric and ε_0 the free space permittivity.

Figure 3 shows the variation of the quality factor for both EM and P modes with different ω_{pe}/ω values. Quality factor Q_p in electron ion model is compared with Q_p of the electron plasma model.

Values of D_e, D_p, Q_e and Q_p are obtained using $h=0.158$ cm, $a=2.1$ cm, $f=2.75$ GHz, $\varepsilon_r=2.31$, $n=1$ and $k_1 a=1.84118$.

DISCUSSION AND CONCLUSION

The present study of different parameters indicates that the presence of plasma media affects the radiation properties to a great extent. Variation of directivity in the EM mode indicates that maximum radiation intensity in a particular direction ($\theta=0°$) decreases with increase in the plasma-to-source frequency. It is maximum in free space ($\omega_{pe}/\omega=0$). In the P mode, the variation of directivity is not uniform. The quality factor curve of EM mode indicates that energy loss decreases for higher ω_{pe}/ω values. For lower values, the quality factor remains almost unchanged. Energy loss in P mode increases with ω_{pe}/ω and becomes infinite at $\omega_{pe}/\omega=1$, making the quality factor zero. The overall conclusion about circular patch antenna is that it is better suited for operation in plasma compared to the linear antennas. However this theory is valid for the range where plasma frequency is lower than the source frequency.

ACKNOWLEDGEMENT

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SYNTHESIS AND CHARACTERIZATION OF TELLURIUM(IV) COMPLEXES WITH 2-NAPHTHOL

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ABSTRACT

Compounds of the general formula $TeCl_{4-n}(OC_{10}H_7)_n$ ($n = 1$–3) have been prepared by reacting tellurium(IV) chloride with 2-naphthol. The compounds act as Lewis acids and form adducts with pyridine, piperidine, γ-picoline, 2, 2′-bipyridyl and 1, 10-phenanthroline. The parent compounds and the adducts have been characterized on the basis of elemental analyses, molar conductance and molecular weight determinations and infrared spectroscopic studies, which indicate the existence of bridged structures through naphthoxy group in the parent compounds and a breakdown of structure of the parent compound on adduct formation.

INTRODUCTION

THE chemistry of metal phenoxides has been a subject of interest because of the catalytic, biological and industrial applications of these compounds[1]. One of the general methods of preparation of phenoxides involves the reaction of metal halides with phenol, wherein OH group is deprotonated and hydrogen chloride gas is evolved. We have already reported the synthesis of phenoxides of Al(III)[2], Ti(IV)[3], Nb(V) and Ta(V)[4] by this method. However, in a recent report[5] on the reaction of tellurium(IV) chloride with phenols, it is claimed that it is not the phenolic proton which takes part in the reaction but that, instead, the proton of the phenol ring is responsible for the evolution of HCl, thereby leading to the formation of hydroxyphenyltellurium(IV) halides. These observations prompted us to undertake the parallel reaction of tellurium(IV) chloride with 2-naphthol and characterize the reaction products.

EXPERIMENTAL

Materials

2-Naphthol (BDH) was recrystallized twice from hot carbon tetrachloride. The sample with melting point 121–122°C was used for various reactions. Tellurium(IV) chloride (Reidel) was used as such without further purification. All the bases were purified by standard methods before use.

Preparation of the complexes

The compounds, which may be formulated as $TeCl_3(OC_{10}H_7)$, $TeCl_2(OC_{10}H_7)_2$ and $TeCl(OC_{10}H_7)_3$, were prepared by mixing the xylene solutions of tellurium tetrachloride and 2-naphthol in appropriate molar ratios under anhydrous conditions, followed by refluxing on an oil bath till evolution of hydrogen chloride gas ceased. The resulting solution was filtered to remove a very small amount of solid formed during the reaction. Addition of an inert solvent like petroleum ether to the solution resulted in the separation of a solid compound, which was filtered and recrystallized from an ether–THF mixture (2 : 1).

The adducts with nitrogenous bases were prepared by stirring the xylene solution of the parent naphthoxide with a base (1 : 1) for about 24 h. Solid compound in each case was isolated by the addition of petroleum ether, and was filtered, washed with petroleum ether, and dried *in vacuo*.

The complexes were analysed for tellurium a tellurium metal, and chlorine was estimated b Volhard's method. Measurements of conductanc were carried out on an Elico conductivity bridg type CM-82T. Infrared spectra of the complexe were recorded in potassium bromide pellets o Perkin Elmer 337 and 621 spectrometers. Molecul weights of all the complexes were determined b cryoscopic method.

RESULTS AND DISCUSSION

The analytical data for the complexes, along wi their colour, molar conductances and molecul weights, are given in table 1. These compounds a quite stable in dry air but change their colour wh

Niobium (V), tantalum

Table 1 *Analytical and IR spectroscopic data for synthesized tellurium (IV) naphthoxides*

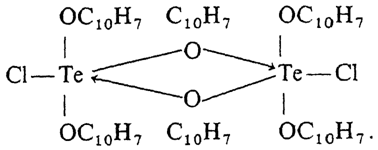
Compound	Colour	Found (calcd), % Te	Found (calcd), % Cl	Λ_M (ohm^{-1} cm^2 mol^{-1})	Molecular weight, found (calcd)	IR ν_{C-O}	IR ν_{Te-O}	IR $\mathrm{Te}\overset{\mathrm{O}}{\longrightarrow}\mathrm{Te}$	IR ν_{Te-Cl}
$TeCl_3(OC_{10}H_7)$	Dirty green	33.93 (33.90)	28.25 (28.21)	9.1	745 (377.5)	1200	—	518, 475	368
$TeCl_2(OC_{10}H_7)_2$	Dirty green	26.38 (26.39)	14.66 (14.64)	8.3	980 (485)	1208	575	525	365
$TeCl(OC_{10}H_7)_3$	Light brown	21.64 (21.60)	5.95 (5.99)	6.9	1176 (592.5)	1212	570	520	360, 345

exposed to moist air. Melting points of these compounds could not be determined correctly because of their high sensitivity to moisture. They are fairly soluble in common organic solvents, such as benzene, nitrobenzene, carbon tetrachloride, chloroform, etc. The molar conductance values of millimolar (10^{-3} M) solutions of the compounds in nitrobenzene show that they are slightly dissociated in solution. Molecular weight determinations cryoscopically in nitrobenzene indicate their molecular complexity to be around two, suggesting dimeric character.

Evolution of hydrogen chloride gas during the preparation of these compounds has been attributed to deprotonation of the phenolic OH group rather than to the action of the ring proton, as evidenced by the complete absence of any band in the region 3380–3600 cm^{-1} assigned to ν_{OH} in pure naphthol[6] and substantiated by the preliminary negative test of phenolic OH group in organic chemistry. There is a significant lowering of ν_{C-O} observed at 1260 cm^{-1} in pure naphthol, by about 50–60 cm^{-1} (table 1), suggesting bonding of oxygen to the metal, which is further confirmed by the appearance of entirely new bands in the region 560–580 cm^{-1} and 480–520 cm^{-1} assigned to the terminal ν_{Te-O}[7] and bridging $\mathrm{Te}\overset{\mathrm{O}}{\longrightarrow}\mathrm{Te}$ modes[8] respectively. In the case of $TeCl_3(OC_{10}H_7)$, however, only bands due to the bridging $\mathrm{Te}\overset{\mathrm{O}}{\longrightarrow}\mathrm{Te}$ modes could be observed. Apart from these bands due to the tellurium–oxygen bond, sharp bands in the region 350–370 cm^{-1} can be assigned to the ν_{Te-Cl} modes[9–11]. No band that could be assigned to the bridging $\mathrm{Te}\overset{\mathrm{Cl}}{\longrightarrow}\mathrm{Te}$ mode expected to fall well below 300 cm^{-1} has been observed, thus suggesting that bridging in these compounds is through naphthoxy groups and not through chlorines. All important bands are given in table 1.

Apart from these bands, it is quite significant that no band was observed in the region 200–250 cm^{-1}, which otherwise could have been assigned to ν_{Te-C} modes[12], thus excluding the formation of organometallic hydroxyphenyltellurium (IV) halides. These observations are in contrast to those of Khandelwal *et al.*[5], who have reported involvement of ring proton and not of phenolic OH in evolution of HCl gas in the reaction of phenol with $TeCl_4$ thereby giving hydroxyphenyltellurium (IV) halides. But we are aware, in view of limited evidence, that the possibility of formation of hydroxynaphthyltellurium (IV) chloride cannot be completely ruled out, because there was always a very small amount of solid left in the solution after the reaction was over, which could not be analysed properly because of limited resources and was therefore rejected.

Thus, based on limited IR spectroscopic observations coupled with conductance and cryoscopic studies, dimeric structures bridging through naphthoxy groups may be proposed. The structure, of $TeCl(OC_{10}H_7)_3$ may therefore be written as



Similar structures may be written for other compounds.

The five-atom coordination around tellurium attained through naphthoxy bridges has changes to six-atom coordination when these naphthoxides are allowed to react with strong bases such as pyridine, piperidine, γ-picoline, 2,2′-bipyridine and 1,10-phenanthroline. These bases react exothermally with the naphthoxides of tellurium (IV) forming compounds of composition $TeCl_{4-n}(OC_{10}H_7)_n.2L$ and $TeCl_{4-n}(OC_{10}H_7)_n.B$ with monodentate (L) and bidentate

Table 2 *Analytical data for the adducts of tellurium (IV) naphthoxides*

Compound	Colour	Found (calcd), %		Λ_M (ohm^{-1} cm^2 mol^{-1})	Molecular weight, found (calcd)
		Te	Cl		
$TeCl_3(OC_{10}H_7).2$ py	Dirty green	23.92 (23.90)	19.83 (19.88)	15.2	528 (535.5)
$TeCl_3(OC_{10}H_7).2$ pip	Light green	23.33 (23.37)	19.39 (19.45)	12.6	537 (547.5)
$TeCl_3(OC_{10}H_7).2$ γ-pic	Light brown	22.69 (22.71)	18.93 (18.89)	14.1	569 (563.5)
$TeCl_3(OC_{10}H_7)$.bipy	Light green	23.93 (23.94)	19.92 (19.96)	16.3	539 (533.5)
$TeCl_3(OC_{10}H_7)$.phen	Light green	22.99 (22.95)	19.07 (19.10)	16.9	552 (557.5)
$TeCl_2(OC_{10}H_7)_2.2$ py	Light green	19.88 (19.90)	11.08 (11.04)	13.2	642 (643)
$TeCl_2(OC_{10}H_7)_2.2$ pip	Light green	19.58 (19.54)	10.79 (10.83)	11.7	658 (655)
$TeCl_2(OC_{10}H_7)_2.2$ γ-pic	Light brown	19.12 (19.07)	10.55 (10.58)	12.5	622 (671)
$TeCl_2(OC_{10}H_7)_2$.bipy	Light green	20.00 (19.96)	11.11 (11.07)	—	648 (641)
$TeCl_2(OC_{10}H_7)_2.2$ phen	Light green	19.30 (19.24)	10.68 (10.67)	—	671 (665)
$TeCl(OC_{10}H_7)_3.2$ py	Dirty green	17.00 (17.05)	4.70 (4.73)	11.0	744 (750.5)
$TeCl(OC_{10}H_7)_3.2$ pip	Light green	16.72 (16.78)	4.68 (4.65)	10.8	763 (762.5)
$TeCl(OC_{10}H_7)_3.2$ γ-pic	Light brown	16.49 (16.44)	4.52 (4.56)	9.7	771 (778.5)
$TeCl(OC_{10}H_7)_3$.bipy	Light green	17.03 (17.10)	4.75 (4.74)	13.2	756 (748.5)
$TeCl(OC_{10}H_7)_3$.phen	Light green	16.59 (16.56)	4.64 (4.59)	13.4	766 (772.5)

(B) ligands respectively, as evidenced by the elemental analysis data given in table 2. Molar conductance values of millimolar solutions of these compounds in nitrobenzene are comparable with the values reported for 1:1 electrolytes. Interestingly, the values of conductance of these addition compounds are a little higher than those of the parent naphthoxides, which suggests that the coordination of the additional monodentate or bidentate nitrogen ligand promotes ionization of the tellurium–halogen bond, thereby causing an increase in conductance. The molecular weights of these adducts in nitrobenzene suggest that they are nearly monomeric.

Coordination of the ligands to the central metal has been ascertained from the shifts of the $\nu_{C=C}$ and $\nu_{C=N}$ modes[13-16], observed usually in the region 1400–1600 cm^{-1} in the pure ligands, towards higher regions on adduct formation, in agreement with the earlier reports. Coordination of piperidine to tellurium was indicated by lowering of the band present at 3291 cm^{-1} due to ν_{N-H} by 50–60 cm^{-1} on adduct formation[13]. Another significant band at 822 cm^{-1}, due to the H–N–C deformation mode[10], is shifted to higher regions, by about 30–40 cm^{-1}. The appearance of new bands in the region 270–280 cm^{-1} in these adducts may be assigned to the $\nu_{Te\text{-}N}$ modes, which confirms the coordination of nitrogen of the ligand to the metal.

In the IR spectra of these adducts, it is significant that bands due to the bridging $\mathrm{Te}\overset{\mathrm{O}}{\longrightarrow}\mathrm{Te}$ modes in the parent naphthoxides disappear on adduct formation and, instead, sharp bands in the region 540–580 cm^{-1} assigned to the terminal $\nu_{Te\text{-}O}$ modes are observed. This suggests that there is a rupturing of naphthoxy bridging followed by coordination of the ligand, forming hexacoordinated tellurium compounds. Similar observations have been made earlier also in the case of adducts o phenoxides of niobium(V) and tantalum(V) with nitrogenous bases[4].

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ANNOUNCEMENTS

Training Course in Ethnobotany

Place: Lucknow
Date: March 1990
Contact: Dr S. K. Jain
Course Director
National Botanical Research Institute
Lucknow 226 001

2-Day Scientific Communication Teaching Courses

Place: Patna
Date: 17–18 March 1990
Contact: Dr B. K. Agarwal
Organizing Secretary
Dept of Gastroenterology
IGIMS, Sheikhpura
Patna 800 014

Place: Srinagar
Date: 20–21 March 1990
Contact: Dr A. H. Durrani
Organizing Secretary
Dept of Medicine
Srinagar Medical College
Srinagar 190 010

International Symposium on Bioseparations

Place: Madras
Date: 11–13 December 1989
Contact: Dr Kunthala Jayaraman
Centre for Biotechnology
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SHORT COMMUNICATIONS

SEARCH FOR A POSSIBLE NEW NEUTRAL COSMIC RAY

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THE experiments of Cowan *et al.*[1-6], if true, suggest the existence of a new electrically neutral cosmic radiation[7] capable of producing a muon in a local absorber. We have carried out an experiment similar to that of Cowan *et al.*[1-3] to verify whether such a radiation exists. Following Cowan *et al.* we have looked for decaying muons in a liquid scintillator tank covered by a shield of plastic scintillators in anticoincidence (figure 1). A concrete shielding provided an aperture or slit of ± 15° in the east-west direction. The absorption of the radiation in the concrete of thickness 400 g/cm^2 is thought to provide a collimating effect to the radiation and depending on the sources in the sky contributing to this radiation there would appear a sidereal time variation in the counting rates.

The experimental arrangement, shown in figure 1, has been set up in the premises of the Tata Institute of Fundamental Research (sea level). It consists of a liquid scintillator tank 100 × 50 × 30 cm filled with 125 l of aviation turbine fuel (kerosene) with dissolved DPO (3 g/l) and wavelength shifter POPOP (5 mg/l). The liquid scintillator is watched by two (denoted as A and B) 12.5 cm photomultiplier tubes (Philips XP2050) in coincidence. The liquid scintillator tank has an anticoincidence cover of plastic scintillators (C) on all sides except the bottom. The plastic scintillators are in modules of 50 × 50 × 5 cm each viewed by a 5 cm photomultiplier (Philips 2212 B) in contact at the middle of the module. This detector system is housed inside a 12.5 cm thick (400 g/cm^2) concrete enclosure having an opening of ± 15° in the zenith in the east-west direction. The shielding acts as a collimator for the cosmic rays.

The events recorded are those having a pair

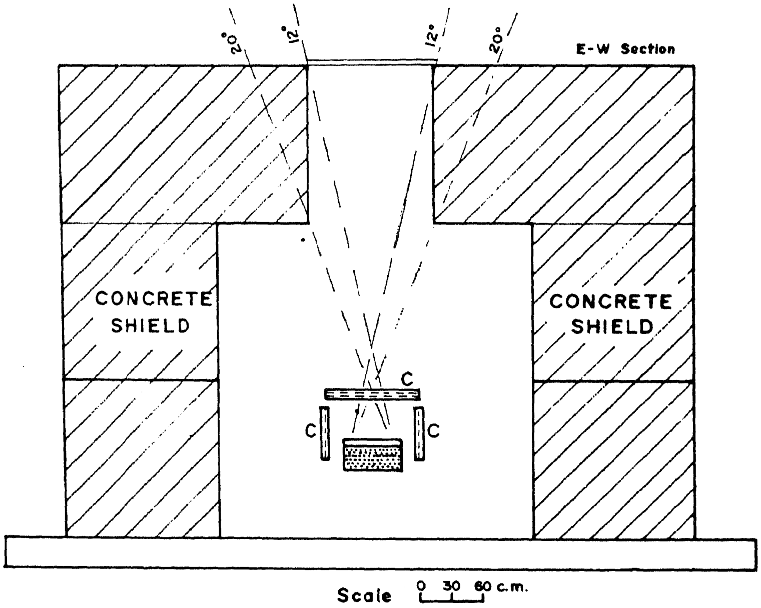

Figure 1. An east-west section of the detector assembly and shielding. C is the anticoincidence plastic scintillator shield for the central liquid scintillator shown dotted.

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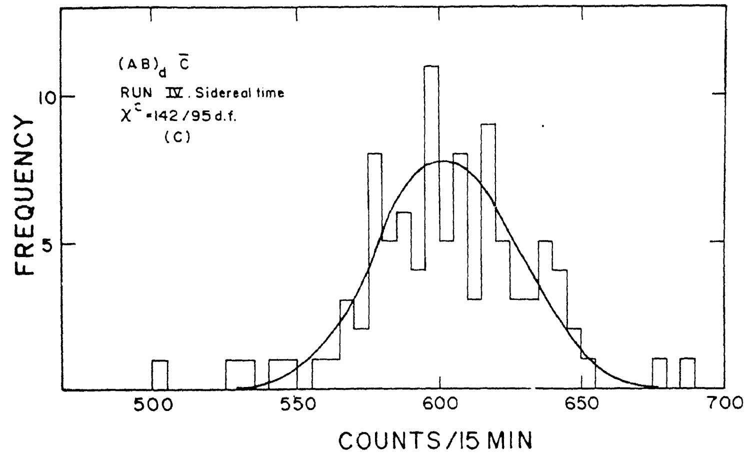

Figure 2. A projection of the sidereal time rate plot of a data set covering 21 days running time. The curve is the Gaussian expected for purely random variation.

(doublet) of coincidence pulses from the photomultipliers A, B from the liquid scintillator within a time separation in the interval of 0.6 to 8 μs with no pulse in the scintillator C. These signals we denote as (AB)$_a\bar{C}$. These events can be construed as having been caused by electrically neutral particles incident on the liquid scintillator and producing a muon decay event in it. The doublet pair spacing distribution shows an exponential with a mean value consistent with 2.2 μs which is the characteristic lifetime of muon.

The (AB)$_a\bar{C}$ events occurring at the rate of a few/minute as well as (AB)$_a$C which corresponds to slow cosmic ray muons stopping and decaying in the tank have been recorded for one or more full days. The data show largely statistical fluctuations when plotted as a 15-minute rate vs local time. The aim of the experiment was to see whether there are fluctuations beyond the statistical when the rate plots are made against sidereal time obtained by shifting the local time by ±4 min a day, the minus sign for following days with respect to a fixed day and the plus sign for preceding days with respect to the same fixed day (chosen as May 15, 1987).

Counting rate distribution obtained in sidereal time (figure 2) is compared with the Gaussian or normal distribution expected from the mean of the distribution. The deviations from the expected curve are barely significant. There are two or three rates occurring outside the Gaussian distribution.

From the results obtained it is difficult to conclude that there is evidence for a new type of neutral cosmic radiation as envisaged. The sidereal effect is marginal though it is at a 3σ level of deviation in significance (figure 2). The level of flux times cross-section per nucleon of the signal if any can be set at 3.8×10^{-29} s^{-1} st^{-1}. This level is much higher than the level of 3×10^{-33} s^{-1} st^{-1} per target nucleon for the signal claimed by Cowan *et al.*[5] An attempt is being made to improve the signal to noise in our detection system.

The authors thank Prof. B. V. Sreekantan for his keen interest. Diligent technical assistance provided by J. V. Shah, J. D. Kulkarni, M. Visagan and N. Jain is also acknowledged.

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A MODEL STUDY OF EFFECT OF ELECTRON TEMPERATURE ENHANCEMENTS ON D- AND E-REGION ELECTRON DENSITIES

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IMMENSE heating of the ionosphere due to absorption when irradiated by high-power, high-frequency radio waves raises the temperature in the region. The consequent (and corresponding) rise in the electron neutral particle collisional frequency is believed to be one of the causes of the observed changes in propagation parameters of LF/VLF waves passing through the disturbed ionosphere. It is generally believed that electron density does not get modified because of the short duration of the heating pulse and the long time-scales required to affect chemical reactions. However, from partial reflection measurements and model calculations, Utlaut and Violette[1] observed that the electron density profile, along with collision frequency, get modified by the heater to different degrees at different altitudes. They also suggest the atmospheric chemistry reaction times are modified. Holway and Meltz[2] suggested that in the D-layer, ohmic heating produces an increase in electron density owing to a decrease in the rate of dissociative recombination. Although there is no direct experimental evidence for this effect, absorption measurements[1, 3] have clearly shown that electron density enhancements are possible following sustained heating. This electron density modification is due to a change in temperature and takes place through temperature-dependent chemical reactions. So a study of the behaviour of electron density profiles under enhanced temperature conditions is of great significance for LF/VLF communications, particularly when high-power medium or high-frequency transmitters are operating in their propagation path. In this note we present the results of such an investigation using a model-derived electron density profile relevant to low latitudes.

The electron density profile

The altitude distribution (60–120 km) of electron density appropriate to a moderate solar activity and winter noontime conditions at low latitudes is considered in this study. The neutral atmosphere model, including the minor species, necessary to obtain ion production rates, is adopted from Solomon *et al.*[4] The required solar ionizing radiation and GCR fluxes are taken from recent literature. A comprehensive ion-chemical scheme given by Reid[5], which considers minor constituent ions like NO^+ and metastable oxygen and water cluster ions as well as negative ions along with the major ion species O_2^+ and N_2^+, is used to obtain electron densities. A detailed description of the ion-chemical scheme and derivation of electron density is given elsewhere[6]. The electron density profile thus obtained is shown in figure 1.

Effect of temperature enhancement on electron density profiles

There are several reactions in the ion-chemical model assumed whose reaction rates are dependent on electron temperature, for example

$$NO^+ + e \rightarrow N + O = 4.2(-7)(300/T_e)^{0.85}$$

To bring out the influence of temperature rise on the electron density, the electron temperature values used in model development were increased by 30%, since a 30% increase is normally expected in an ionospheric heating experiment[7]. Using the modified electron temperature values, the altitude distribution of electron density is computed as described earlier. The modified electron density profile thus obtained is also shown in figure 1. It is interesting to see that, consequent to a rise in electron temperature, the change in electron density is different in different altitude zones. First there is a small reduction below about 74 km, then a significant increase between 74

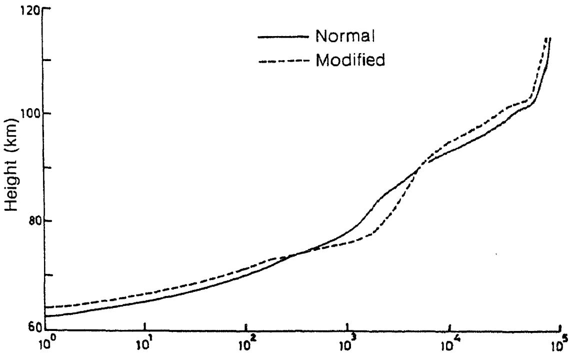

Figure 1. Electron density profile for low latitudes derived from a neutral atmosphere model and ion-chemical scheme.

Ionospheric modification

and 90 km with electron density change maximizing at 80 km, and a decrease again beyond 90 km.

It is not strictly valid to assume a uniform temperature enhancement in an ordinary heating experiment, since the region (altitude) of maximum absorption of radiation (and thereby temperature rise) depends on the frequency of the disturbing transmitter and also the mode of excitation. Nevertheless, the present result is significant in the sense that it brings out the variation in the effect of temperature enhancement on the various chemical processes. It may be of interest to note that the regions of crossover (figure 1) coincide, respectively, with the region of transition from negative ion to electron-dominant regime (at around 74 km) and the region where O^+ ions dominate over NO^+ ions. A detailed investigation using a realistic temperature distribution under disturbed conditions is being carried out and the results will be published elsewhere.

The authors thank UGC and CSIR, New Delhi, for financial assistance.

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POLAROGRAPHIC STUDIES OF MIXED-LIGAND COMPLEXES OF THE In(III)–1-METHYLIMIDAZOLE–TARTRATE SYSTEM

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STUDIES on mixed-ligand complexes, particularly those of biological significance, have been of interest during the past decade. Earlier work from this laboratory and elsewhere[1–3] has indicated that in the combination of metals with serum albumin the principal binding sites are the imidazole groups of the protein molecule. Norman *et al.*[4] have studied polarographically the overall formation constant of cadmium and copper complexes of imidazole and 1-methylimidazole. The results indicated that the site of binding on the imidazole molecule was the pyridine-nitrogen rather than pyrrole-nitrogen. A survey of data cited in the literature revealed that further studies are required. Also, there has been no report on mixed-ligand complexes of In(III)–1-methylimidazole–tartrate system. The present work is devoted to a polarographic investigation of the composition and stability constants of simple and mixed-ligand complexes of the above system.

All chemicals used were of BDH reagent grade. Solutions were prepared in conductivity water. The ionic strength was maintained constant at $\mu = 2.0$ using $NaNO_3$ supporting electrolyte. The concentration of Cd(II) was kept at 1×10^{-3} M. All measurements were made at 25 ± 0.1°C and pH 8. The pH measurements of the solutions were made using a Beckman extended scale digital pH meter model 4500 with accuracy ± 0.002 pH units. The pH meter was calibrated by means of saturated buffer solutions (Burroughs Wellcome, England).

Polarographic measurements were carried out using a PAR model 174A polarographic analyser in conjuction with a three-electrode cell system, with a saturated Ag/AgCl as reference, platinum as the counter electrode and a dropping mercury electrode as working electrode. The polarograms were recorded on Houston Omnigraphic model RE 0074 X–Y recorder. All the test solutions were degassed with pure nitrogen.

The stability constants of simple complexes of In(III) with 1-methylimidazole and tartrate were determined separately prior to a study of the mixed-ligand system. Identical conditions were maintained in both the simple and mixed-ligand systems.

The reduction of In(III) in 1-methylimidazole and tartrate media, separately, was found to be reversible and diffusion-controlled. The same was true with the mixed system. The slopes of linear plots of log $(i/i_d - i)$ vs $E_{d.e.}$ were of the order of 33 mV and the plots of i_d vs $h^{\frac{1}{2}}_{corr}$ were linear and passed through the origin.

In(III)–1-methylimidazole

A plot of $E_{\frac{1}{2}}$ vs log [1-MeIm] indicated that two complexes were formed, with stability constants $\log B_{10} = 3.60$ and $\log B_{20} = 5.86$. Deford and Hume's[5] method was applied to determine the stability constants of successive complexes.

In(III)–tartrate system

The $E_{\frac{1}{2}}$ vs $\log[\mathrm{Tart}^{-2}]$ curve gave a straight line indicating the formation of one complex species and hence the method of Lingane[6] was adopted for the evaluation of stability constants and co-ordination number. The stability constant of the simple complex formed was found to be $\log B = 3.4$.

Mixed In(III)–1-methylimidazole–tartrate system

The 1-methylimidazole concentration was varied from 0.02 to 0.20 M and that of tartrate was kept constant at 0.1 M. The $E_{\frac{1}{2}}$ values were geater than those obtained in the absence of tartrate, thereby indicating the formation of mixed complexes. The system was studied at another concentration of tartrate (0.20 M). To determine the values of the stability constants of mixed-ligand complexes, the method of Schaap and McMasters[7] was applied. The polarographic characteristics and function $F_{ij}(X)$ of the mixed complexes at fixed $[\mathrm{Tart}^{-2}]$ (0.1 and 0.2 M) are presented in tables 1 and 2.

Table 1 *In(III)–1-methylimidazole–tartrate system, [In(III)] = 1 × 10⁻³ M and [Tart⁻²] = 0.1 M (fixed)*

[1-MeIm]	$-E_{\frac{1}{2}}$	$F_{00}(X)\cdot 10^{-1}$	$F_{10}(X)\cdot 10^{-3}$	$F_{20}(X)\cdot 10^{-5}$
0.00	0.510			
0.02	0.520	3.95		
0.04	0.530	21.53	3.98	
0.08	0.540	179.55	22.00	26.59
0.10	0.550	339.00	32.97	28.00
0.15	0.560	1504.98	99.87	41.98
0.20	0.570	2139.99	106.79	24.89

$\mu = 2.0$ $NaNO_3$, pH 7.4, temp. 25°C.

Table 2 *In(III)–1-methylimidazole–tartrate system, [In(III)] = 1 × 10⁻³ M and [Tart⁻²] = 0.2 M (fixed)*

[1-MeIm]	$-E_{\frac{1}{2}}$	$F_{00}(X)\cdot 10^{-1}$	$F_{10}(X)\cdot 10^{-3}$	$F_{20}(X)\cdot 10^{-5}$
0.00	0.510			
0.02	0.530	2.09		
0.04	0.540	6.98	13.31	23.20
0.08	0.550	35.31	42.00	47.56
0.10	0.560	72.00	70.00	58.00
0.15	0.570	339.87	229.00	94.99
0.20	0.580	960.99	279.98	72.98

$\mu = 2.0$ $NaNO_3$, pH 7.4, temp. 25°C.

The stability constants were calculated from the analysis of $F_{ij}(X)$ functions.

The values are reproducible to ±4%. Two mixed complexes as noted below are formed:

$$[\mathrm{In(1\text{-}MeIm)(Tart)}] \qquad \log B_{11} = 4.5$$
$$[\mathrm{In(1\text{-}MeIm)(Tart)_2}]^{2-} \qquad \log B_{12} = 6.0.$$

The value of the mixing constant K_M is +0.7 (ref. 8, 9). The positive value of the mixing constant shows that the mixed complex [In(1-MeIm)(Tart)] is somewhat more stable than the simple biscomplexes of In(III) with 1-methylimidazole and tartrate.

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REACTION OF SOME *p*-SUBSTITUTED PHENACYLDIMETHYLSULPHONIUM BROMIDES WITH AROMATIC ALDEHYDES: SYNTHESIS OF 2,4,6-TRIARYLPYRIMIDINES

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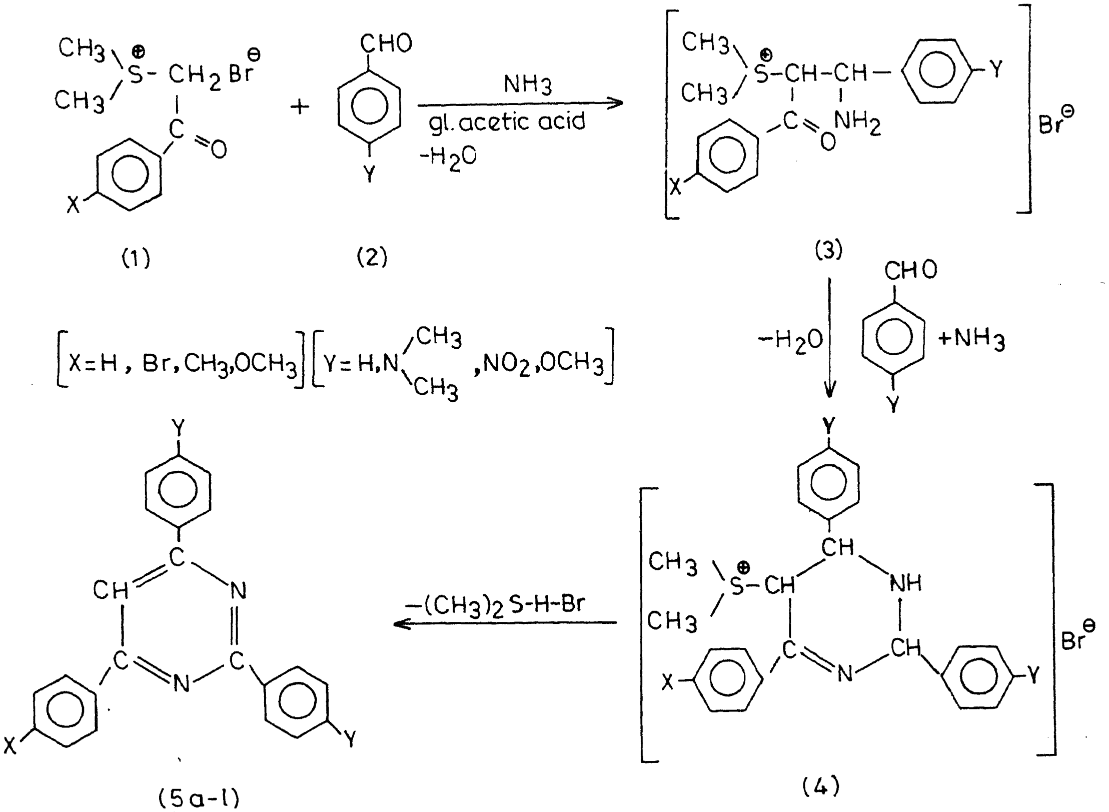
SULPHONIUM salts have gained considerable importance in the synthesis of a large variety of heterocyclic compounds, viz. substituted pyridines[1,2], indoles[3], tetrazines[4], azaridines[5], etc. But they have not been utilized in the synthesis of 2,4,6-triarylpyrimidines. Work described here involves aza ring closure with condensation of sulphonium salts with aromatic aldehydes in the presence of glacial acetic acid and liquid ammonia, leading to the synthesis of 2,4,6-triarylpyrimidines (5a-l).

Heating a mixture of *p*-substituted phenacyldimethylsulphonium bromide with benzaldehyde carrying electron attracting and donating substituents at the *para* position in the presence of glacial acetic acid and liquid ammonia at reflux temperature for 4–5 resulted in 2,4,6-triarylpyrimidine with 40–80% yield (scheme 1).

The course of reaction involves a Mannich-type reaction. The methylene group of the salt (1) with aromatic aldehyde (2) in the presence of liquid ammonia forms a Mannich base–sulphonium salt (3), which, in turn, undergoes condensation with another molecule of benzaldehyde in the presence of ammonia to form a sulphonium salt intermediate (4). The latter then undergoes elimination of dimethylsulphonium hydrobromide and H_2 to form 2,4,6-triarylpyrimidine (5a-l).

Some 2,4,6-triarylpyrimidines (5a-l) synthesized by the above route gave satisfactory elemental and



	X	Y
5a:	4–H	4–H
b:	4–H	4–N(CH₃)₂
c:	4–H	4–NO₂
d:	4–Br	4–H
e:	4–Br	4–NO₂
f:	4–Br	3,4–OCH₃
g:	4–CH₃	4–H
h:	4–CH₃	4–NO₂
i:	4–CH₃	4–N(CH₃)₂
j:	4–OCH₃	4–NO₂
k:	4–OCH₃	4–N(CH₃)₂
l:	4–OCH₃	4–H

Scheme 1.

spectral analyses. The IR spectroscopic data show characteristic absorption bands in the region 3100 to 3000 cm^{-1} which were assigned to C–H stretching mode of pyrimidine ring. The bands in the region 1600 to 1500 cm^{-1} are due to interaction between C=C and C=N vibrations of the ring. The NMR spectra of pyrimidines showed a pyrimidyl proton (C_5–H) in the range δ6.40–6.80 and aromatic protons in the range δ6.60–8.40.

All substituted phenacyldimethylsulphonium bromides (1) were prepared by heating *p*-substituted phenacyl bromide with dimethylsulphide in acetone as reported in the literature[1, 2, 7–9].

Preparation of 2,4,6-triarylpyrimidines (5a-l)

A mixture of 3 mmoles of *p*-substituted phenacyldimethylsulphonium bromide (1), 6 mmoles of aromatic aldehyde (2) and 3 g of ammonium acetate in 50 ml of glacial acetic acid was stirred at room temperature for 6–10 h. The mixture was then refluxed for 4–8 h and left at room temperature overnight. It was then poured into ice-cold water (50 ml) with constant stirring. The solid mass precipitated was filtered and washed twice with water and then with methanol. The product, on crystallization with appropriate solvents, gave crystalline 2,4,6-triarylpyrimidine (5a-l) in 40–80% yield.

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SYNTHESIS OF THIOSEMICARBAZIDES, TRIAZOLES, THIADIAZOLES AND OXADIAZOLES

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A broad spectrum of biological activity, including antibacterial, antifungal, anticonvulsant, fungicidal, anti-inflammatory and tranquilizing effects, is reported to be associated with thiosemicarbazides, triazoles, thiadiazoles and oxadiazoles[1–6]. Therefore it was of interest to synthesize 4,4′-diphenyl-*bis*-pimelyl/suberyl/sebacyl/azelyl thiosemicarbazides (IIa–d), *bis*(4-phenyl-5-mercapto-1,2,4-triazol-3-yl) alkanes (IIIa–d), *bis*-(5-phenylamino-1,3,4-thiadiazol-2-yl) alkanes (IVa–d), and *bis*(5-mercapto-1,3,4-oxadiazol-2-yl) alkanes (Va–d) from pimelic, suberic, sebacic and azelaic acids. Pimelic and azelaic acids were obtained by the oxidation of 7-hydroxyheptanal and azelaic acid aldehyde, the periodate oxidation[7] products of aleuritic acid (9,10,16-trihydroxypalmitic acid), the major constituent acid of shellac.

The thiosemicarbazides IIa–d were obtained by the condensation of methanolic solution of the acid hydrazides (Ia–d) with phenylisothiocyanate at steam-bath temperature.

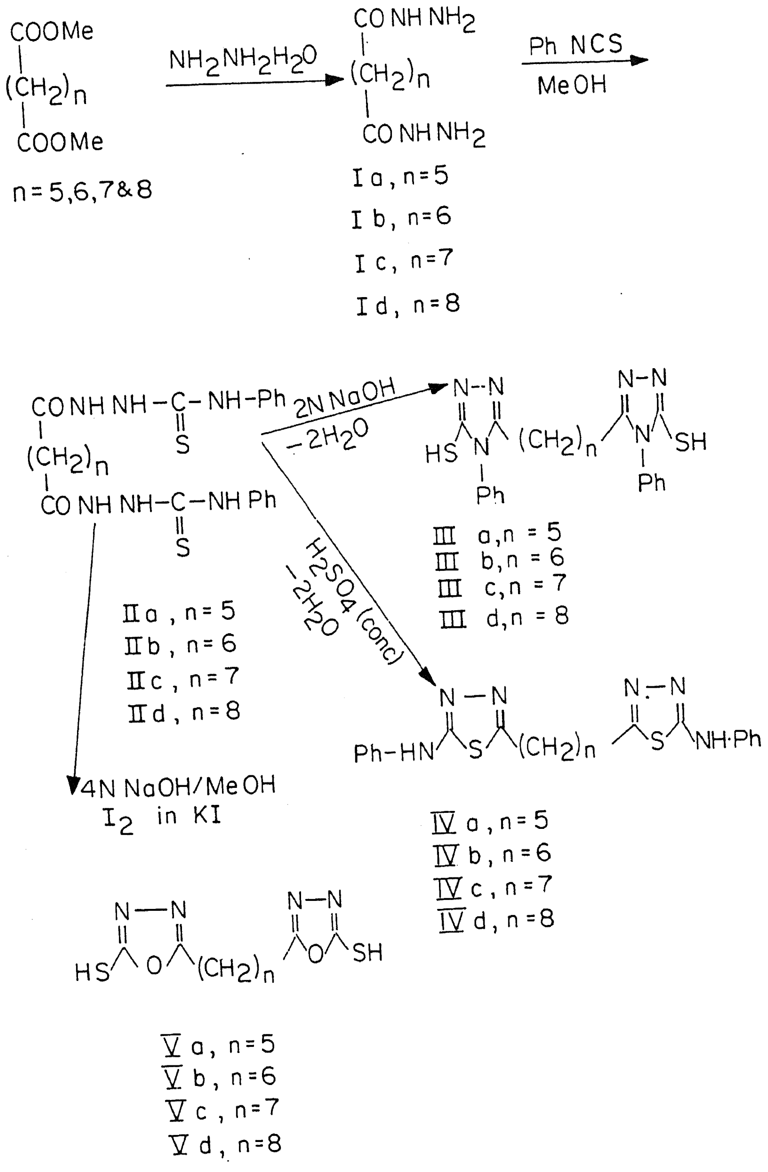
Bis(4-phenyl-5-mercapto-1,2,4-triazol-3-yl) alkanes IIIa–d were obtained by the cyclization of IIa–d with 2 N NaOH at reflux temperature. Treatment of IIa–d with ice-cold conc. H_2SO_4 at room temperature for 2 h afforded *bis*-thiadiazoles IVa–d, while thiosemicarbazide IIa–d in 4 N NaOH when heated under reflux in methanol and iodine in KI solution afforded *bis*-oxadiazoles Va–d.

4,4′-Diphenyl-bis-pimelyl/suberyl/sebacyl/azelyl thiosemicarbazides (IIa–d)

Methanolic solution of acid hydrazide (I, 0.1 mol) and phenylisothiocyanate (0.21 mol) was refluxed for 4 h. It was then cooled, and the solid that separated out was filtered, washed, dried and crystallized. Physical data are reported in table 1.

Bis(4-phenyl-5-mercapto-1,2,4-triazol-3-yl) alkane (IIIa–d)

These compounds were prepared according to th method of Mishra and Bahel[8]. The characterizatio data are given in table 2.



Scheme

Bis(5-phenylamino-1,3,4-thiadiazol-2-yl) alkanes (IVa–d)

The titled compounds were prepared following the procedure reported in the literature[8]. The data are reported in table 3.

Bis(5-mercapto-1,3,4-oxadiazol-2-yl) alkanes (Va–d)

Thiosemicarbazide (1 g) in 4 N NaOH (5 ml) was heated under reflux in methanol (25 ml) and then iodine (2.5 g) in 10 ml of KI (3.2 g) solution was added to it till the colour of iodine persisted. The

Table 1 *Physicochemical data for thiosemicarbazides*

Compound	*n*	Yield (%)	m.p.* (°C)	Mol. formula	Analysis	(%)
					N Found (Cald.)	S Found (Calcd.)
IIa	5	80	87–89	$C_{21}H_{26}O_2N_6S_2$	18.21 (18.34)	14.00 (13.97)
IIb	6	70	177–78	$C_{22}H_{28}O_2N_6S_2$	17.62 (17.79)	13.51 (13.55)
IIc	7	75	172–73	$C_{23}H_{30}O_2N_6S_2$	17.22 (17.28)	13.00 (13.16)
IId	8	72	92–93	$C_{24}H_{32}O_2N_6S_2$	16.78 (16.80)	12.62 (12.80)

*All the compounds were crystallized from MeOH.
IR (KBr): 3200–3190 (–NH), 1680–1675 (secondary-CONH), 1180–1175 (–C=S) cm^{-1}.

Table 2 *Physicochemical data for triazoles*

Compound	*n*	Yield (%)	m.p.* (°C)	Mol. formula	Analysis	(%)
					N Found (Requires)	S Found (Requires)
IIIa	5	75	140–42	$C_{21}H_{22}N_6S_2$	19.84 (19.90)	14.92 (15.16)
IIIb	6	70	243–45	$C_{22}H_{24}N_6S_2$	19.20 (19.26)	14.60 (14.67)
IIIc	7	68	228–30	$C_{23}H_{26}N_6S_2$	18.56 (18.66)	13.93 (14.22)
IIId	8	60	102–3	$C_{24}H_{28}N_6S_2$	17.90 (18.10)	13.70 (13.79)

*The compounds were crystallized from EtOH.
IR (KBr): 3200–3150 (–NH), 1500–1490 (–C=N), 1180–1175 (C=S) cm^{-1}.
PMR: δ 7.3 (*s*, NH, Ar–H), 2.15–3.15 (*t*, methylene protons).

Table 3 *Physicochemical data for thiadiazoles*

Compound	*n*	Yield (%)	m.p.* (°C)	Mol. formula	Analysis	(%)
					N Found (Requires)	S Found (Requires)
IVa	5	70	87–89	$C_{21}H_{22}N_6S_2$	19.81 (19.90)	14.95 (15.16)
IVb	6	68	226–28	$C_{22}H_{24}N_6S_2$	19.00 (19.26)	14.53 (14.67)
IVc	7	65	183–85	$C_{23}H_{26}N_6S_2$	18.50 (18.66)	14.20 (14.22)
IVd	8	63	105–6	$C_{24}H_{28}N_6S_2$	17.92 (18.10)	13.72 (13.79)

*Solvent used for crystallization was MeOH.
IR (KBr): 3320–3300 (secondary-NH), 1500–1490 (conjugated cyclic system –C=N), 1175–1170 (–C=S) cm^{-1}.
PMR: δ 6.5–6.7 (*s*, –NH), 7.1–7.7 (*m*, Ar–H).

Table 4 *Physicochemical data for oxadiazoles*

					Analysis	(%)
Compound	*n*	Yield (%)	m.p.* (°C)	Mol. formula	N Found (Requires)	S Found (Requires)
Va	5	68	240(d)	$C_9H_{12}O_2N_4S_2$	20.60 (20.58)	22.90 (23.52)
Vb	6	65	233–35	$C_{10}H_{14}O_2N_4S_2$	19.38 (19.58)	22.30 (22.37)
Vc	7	58	180	$C_{11}H_{16}O_2N_4S_2$	18.45 (18.66)	21.25 (21.33)
Vd	8	60	112–13	$C_{12}H_{18}O_2N_4S_2$	17.63 (17.83)	20.20 (20.38)

*Compounds crystallized from EtOH.
IR (KBr): 3150–3100 (–NH), 1510–1500 (–C=N), 1175–1170 (C=S) cm^{-1}.

mixture was then concentrated and cooled, and the solid which separated was filtered, dried and crystallized. The data are given in table 4.

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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF THIOMALATES OF Ag(I), Hg(II) AND Cd(II)

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THIOMALIC acid (TMA) is a versatile ligand with a 'soft' sulphur of a sulphhydryl group and 'hard' oxygens of two carboxylate groups. Its complexes with many metal ions have been reported in solution[1–6], though only a few of them have been isolated and characterized[7–10]. TMA is also a biologically important compound which has been shown to serve as an antidote against cadmium, mercury and arsenic poisoning[11]; its complexes with some metal ions have been recommended in the treatment of various disorders[12–14]. In this context it was thought desirable to synthesize and characterize some more complexes and also determine their antimicrobial activity. In this paper such studies on metal thiomalates of Ag(I), Hg(II) and Cd(II) are reported.

Metal acetates and nitrates and thiomalic acid used were of analytical reagent grade. IR spectra (4000–200 cm^{-1}) were recorded on a NICOLET-DX in KBr–CsI pellet/nujol mull. ^1H NMR spectra were recorded in a JEOL FT NMR Fx 90Q spectrometer in DMSO-d_6.

Thermogravimetric analysis was carried out on a fabricated instrument. Heating rate was maintained at 10°C/minute.

Preparation of metal thiomalates

$Ag_2[C_4H_4O_4S]$: Isolation of this 2:1 Ag(I)–TMA complex has been reported by Ellis *et al.*[7] However, a different method was employed to obtain better yield. Methanolic solution of TMA (0.01 mol) was added to methanolic solution of $AgNO_3$ (0.02 mol) dropwise with constant stirring. This resulted in the precipitation of an amorphous white powder. It was filtered, washed with methanol and then with ether, and dried over $CaCl_2$ *in vacuo*. The yield was 83%. The compound decomposes without melting at ~140°C.

$Ag[C_4H_5O_4S]$: 1:1 Ag(I)–TMA complex, hitherto reported only in solution phase[7], was isolated as follows: $AgNO_3$ (0.02 mol) and TMA (0.02 mol) were dissolved in 100 ml and 10 ml of methanol respectively. TMA solution was added to $AgNO_3$ solution dropwise with constant stirring. A white precipitate appeared first, which dissolved afterwards, yielding a yellow solution. This solution was concentrated to ~30 ml in vacuum over P_2O_5. Petroleum ether was added dropwise with stirring. A light yellow solid separated. The material was filtered, washed with a mixture of acetone and petroleum ether (1:9), and dried over $CaCl_2$ *in vacuo*. The yield was 60%. The compound decomposes without melting at ~115°C.

Cd(II) thiomalate and Hg(II) thiomalates: Complexes of 1:1 stoichiometry were prepared by the method of Patil and Krishnan[8]. Another Hg(II) complex, of 1:2 stoichiometry, reported in solution only[1, 15, 16] was isolated as follows: 0.01 mol of $Hg(CH_3COO)_2$ and 0.02 mol of TMA were dissolved in 10 ml and 50 ml of methanol respectively. The mercuric acetate solution was added to TMA solution with constant stirring. The clear solution that resulted was concentrated to about 25 ml *in vacuo*. Benzene (about 15 ml) was added with constant stirring to separate a white product. This was filtered, washed with a mixture of benzene and methanol (80:20), and dried *in vacuo* over P_2O_5. The yield was 70%. The compound decomposes without melting at ~170°C.

Analysis

Ag(I) was determined volumetrically by Volhard's method[17a], and Hg(II) gravimetrically as sulphide[17b] after decomposing the complexes with concentrated HNO_3. Sulphur was estimated 17c as $BaSO_4$ after decomposing the complex with Na_2CO_3 and Na_2O_2. Carbon and hydrogen contents were estimated microanalytically on a CARLO ERBA model 1106 at RSIC, Chandigarh. The analytical data are given in table 1.

IR and ^1H NMR spectroscopic studies

The S–H stretching mode of TMA occurring near 2560 cm^{-1} disappears in all the thiomalates, indicating the linkage of Ag(I) or Hg(II) with S of the sulphhydryl group. This is supported by C–S stretching frequencies of these complexes, which are shifted towards lower frequency (i.e. $< 660\ cm^{-1}$). The thiomalates show a strong band at 1680 cm^{-1} which is due to free –COOH indicating the presence of at least one such –COOH group in all the compounds. Actually, in the case of $Ag[C_4H_5O_4S]$, both the carboxylic groups appear to be free on the basis of ^1H NMR. The ^1H NMR spectrum of this complex clearly shows the presence of two carboxylic protons centred at δ 9.7 ppm. The –CH– proton appears as a triplet at δ 3.9 ppm, and two $-CH_2-$ protons as a multiplet overlapping with peaks of impurity in DMSO–d_6 at δ 2.8 ppm.

However, in $Ag_2[C_4H_4O_4S]$, the second silver atom may be coordinated to one of the carboxylate groups. A strong band around 1560 cm^{-1} reflects the coordination of this group[8] to Ag(I). Thus, in this complex, the TMA moiety acts as a bridge between two silver atoms. ^1H NMR spectra of this compound and $Hg[C_4H_5O_4S]_2$ could not be recorded because of their insolubility in common solvents. However, the presence of all the four –COOH groups in the free state was inferred by titrating this compound against NaOH; four moles of alkali were consumed when one mole of aqueous suspension of the complex was titrated against NaOH. This confirmed that mercury is bonded to both the S atoms of the sulphhydryl groups of two TMA molecules. The ν_{M-S} in these complexes is observed in the range 350–200 cm^{-1} as a rather weak and broad band.

Table 1 *Analytical data for TMA complexes*

	% Found (% calc.)			
Complex	Carbon	Hydrogen	Sulphur	Metal
$Ag_2[C_4H_4O_4S]$	14.23 (13.19)	1.19 (1.10)	14.78 (13.73)	59.29 (59.29)
$Ag[C_4H_5O_4S]$	18.35 (18.68)	1.94 (1.95)	12.23 (12.45)	41.14 (41.97)
$Hg[C_4H_5O_4S]_2$	19.32 (19.32)	2.01 (2.01)	11.73 (12.90)	41.67 (40.37)

Table 2 *Antimicrobial activity of TMA and metal thiomalates*

Compound	MIC (μg/ml)			
	S. aureus	*P. aeruginosa*	*E. coli*	*C. albicans*
TMA	1000	500	500	*
$Ag_2[C_4H_4O_4S]$	250	250	250	500
$Ag[C_4H_5O_4S]$	*	*	*	*
$Cd[C_4H_4O_4S]$	250	250	250	500
$Hg[C_4H_4O_4S]$	**	**	**	**
$Hg[C_4H_5O_4S]_2$	**	**	125	250

*Active as neat samples only.
**Inactive.

A broad absorption (3480–2600 cm^{-1}) due to OH stretching and the presence of submaxima of varying intensity suggest polymeric nature for the compounds due to hydrogen bonding between free –COOH groups of neighbouring molecules[18, 19]. However, the asymmetric and symmetric C–H stretch vibrations ($\sim 2900\ cm^{-1}$) of methylene and methine groups are also expected to overlap in this very region.

Formation of Ag_2S and HgS was found in thermogravimetric analysis of Ag(I) and Hg(II) thiomalate respectively. This supports the conclusion drawn from IR studies that the metal atoms are linked to the S atom of the sulphhydryl group.

Molecular weight and conductance measurements could not be carried out because of the low solubility of the complexes in common solvents.

Antimicrobial activity

TMA and its complexes were tested[20] for antimicrobial activity against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and a fungus (*Candida albicans*). The minimum inhibitory concentrations (MIC) are listed in table 2. The data give a clear indication that antimicrobial activity of TMA is appreciably enhanced after complexation with metal ions.

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INHERITANCE OF SALT TOLERANCE IN PROGENIES OF TISSUE CULTURE SELECTED VARIANTS OF RICE

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TISSUE culture is now being widely employed to select for stress-tolerant variants in crop plants[1-4]. However, the variants generated by this technique are not always true-breeding, as they could have arisen from epigenetic or physiological causes[5]. Such variants may find no use in crop improvement and therefore it is important to identify true genetic mutants. This can be done by studying the stability of the altered trait through a few sexual generations[6,7]. In the present study 221 rice regenerants (R_0) were generated from control and stressed calli through somatic embryogenesis. The inheritance of salt tolerance in the first seed progeny (R_1) is reported in this paper.

Calli were induced from mature embryos of the salt susceptible varieties IR20 and IR50 in induction medium (MS (ref. 8) +2 mg/l 2,4-D+0.5 mg/l kinetin). The calli were then taken through six passages, each passage lasting 3–4 weeks, in salinized medium (MS+2 mg/l 2,4-D+0.5 mg/l kinetin+10 g/l NaCl) and control medium (without NaCl). At the end of each passage some of the surviving calli in each variety were put in regeneration medium (MS+2 mg/l kinetin+0.5 mg/l NAA) for regeneration. The regenerants (R_0), after hardening, were grown in pots to maturity, and R_1 seeds collected from them individually. The R_1 progeny lines were raised in seed boxes (100 seeds per row) and when the seedlings were 15 days old, they were salinized with 0.5% NaCl solution. Resistant (Pokkali) and susceptible (IR20) checks were also sown in each seed box for comparison. When all the 'check' seedlings of IR20 were dead (after 5 days), the number of surviving R_1 seedlings was counted in each progeny row. The proportion of resistant to susceptible seedlings, based on seedling mortality, was calculated for each R_1 progeny row.

Of 221 lines, only two, viz. one of IR20, derived from callus stressed for three passages, and the other of IR50, derived from callus stressed for four passages, were found to be completely tolerant at the seedling stage. The appearance of these two variants can be explained by dominant mutations, as has been observed for brown spot tolerance in tissue culture selected rice variants[9]. In addition, 51 lines were found to be segregating for salt tolerance and susceptibility (table 1). In IR20, 20 lines derived from stressed calli and 9 from the control, and in IR50, 12 lines from stressed calli and 10 from the control segregated. All the other R_1 lines evaluated were found to be totally susceptible. Progenies with resistant variants seemed to be produced with higher frequency from the regenerants of stressed calli compared to those from control.

The pattern of segregation in each of the segregating R_1 lines of IR20 and IR50 showed that only 25% or less of the seedlings were resistant and this suggested a recessive bias for the mutation conferring salt tolerance.

None of the lines showed a strict Mendelian pattern of segregation. However, two lines each from

Table 1 *Number of segregating and non-segregating R_1 progenies in the salt tolerance screening trial*

	IR20				IR50			
	Control		Stressed		Control		Stressed	
Number of passages	S	NS	S	NS	S	NS	S	NS
1	1	20	3	14	1	16	1	13
2	1	16	5	6	2	9	2	7
3	1	8	3	5	1	5	1	6
4	3	6	3	4	2	5	3	3
5	2	5	3	3	2	4	2	3
6	1	5	3	3	2	3	3	1
	9	60	20	35	10	42	12	33

S, Segregating; NS, nonsegregating.

Salt resistant varieties

IR20 and IR50 broadly showed a 15:1 segregation (susceptible to tolerant), three lines from IR50 showed 13:3 segregation and another three lines from IR50 showed a 1:1 segregation at the seedling stage (based on survival). The resistant segregants have been transplanted to the field and are being grown to maturity to get the R_2 generation seeds.

Stable salt-tolerant variants have been generated through tissue culture in tobacco and *Citrus*[11]. In rice also, salt-tolerant variants have been generated through cellular level screening, using seawater[12] or NaCl[13,14] as stressing agents. The present study reaffirms the fact that salt-tolerant variants that may find use in crop improvement can be generated through callus level screening in rice. A systematic progeny evaluation would reveal the actual worth of these variants in developing salt-tolerant varieties.

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NEUROANATOMY AND CHOLINESTERASE ACTIVITY IN *ACANTHOTAENIA MULTITESTICULATA* (CESTODA)

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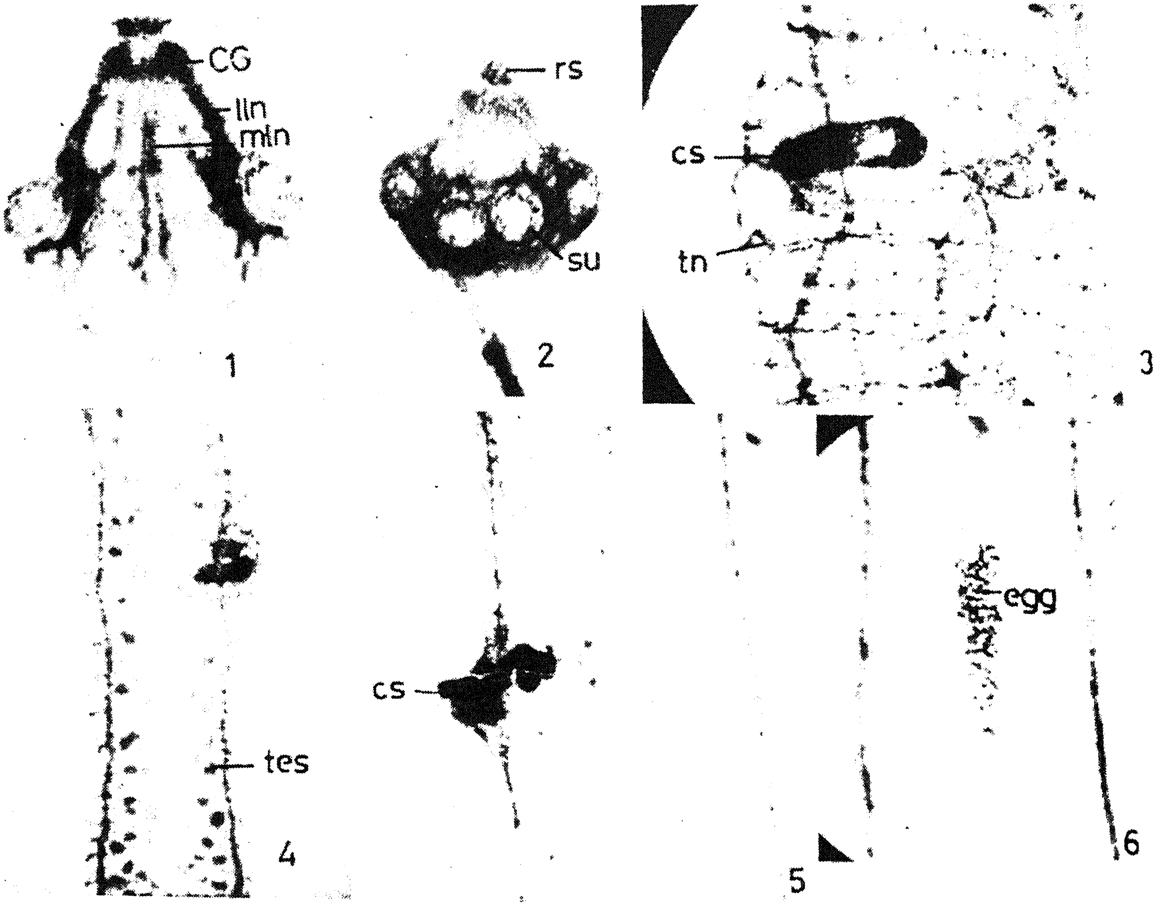
ACETYLCHOLINESTERAE (AChE, EC 3.1.1.7) has been shown in the nervous system in cestodes[1–4], nematodes[5,6] and trematodes[7]. However, comparatively less work has been done on this aspect in cestodes[8]. This communication reports work on AChE localization in the cestode *Acanthotaenia multitesticulata* (Proteocephalidae).

The parasites were collected from the intestine of the monitor lizard *Varanus bengalensis*. Immediately after removal from the host, the worms were washed twice in Tyrode's solution, pressed gently under a coverslip, and fixed in 10% formalin for 20 min. Following fixation the worms were washed in cold distilled water to remove all traces of the fixative. After washing, one group of worms was incubated in the medium of Holt and Withers[4], using 5-bromoindoxyl acetate as substrate for 3–6 h at room temperature to detect non-specific esterases (NSE). Some worms were incubated in acetylthiocholine iodide (AThChI) to localize AChE following the direct colouring method of Karnovsky and Roots[5]. AChE activity was determined by its ability to hydrolyse the above substrates in the presence of pseudocholinesterase inhibitor tetraisopropyl pyrophosphoramide (10^{-5} μM)[9]. The possibility that other esterases besides cholinesterase could hydrolyse the substrates was eliminated in experiments using the specific cholinesterase inhibitor eserine sulphate (10^{-5} μM).

The AChE activity in the parasites and in the medium was assayed colorimetrically. The enzyme assay and *in vitro* studies were carried out following the method of Gunn and Probert[3].

There are two small cerebral ganglia in the scolex, connected by a short cerebral commissure (figure 1). The two ganglia give rise to two median longitudinal



Figures 1–6. Localization of acetylcholinesterase and non-specific esterase activity in *Acanthotaenia multitesticulata*. **1,** AChE activity showing distribution of nerves in the scolex region (after incubation in acetylthiocholine iodide). **2,** NSE activity in scolex (after incubation in 5-bromoindoxyl acetate). **3,** AChE activity in a mature segment, showing distribution nerves. **4,** AChE activity in mature segment, showing enzyme activity in the testes. **5,** NSE activity in the cirrus sac. **6,** NSE activity in embryos in gravid segment. cg, Cerebral ganglion; lln, lateral longitudinal nerve; mln, median longitudinal nerve; rs, rostellum; su, sucker; cs, cirrus sac; tn, transverse nerves; tes, testes.

nerves and continue posteriorly. A much thicker prominent nerve, the laterodorsal, and two thinner lateroventral nerves originate distally from each ganglion and proceed posteriorly on either side of each median longitudinal nerve. In the gravid segments the dorsal longitudinal nerves are prominent, but median and ventral longitudinal nerves are not prominent (figure 6). The suckers are innervated with fine branches of nerves arising from the cerebral ganglia. The longitudinal nerves are connected by transverse nerves separating each segment (figure 3). The rostellar hooks of *A. multitesticulata* showed both AChE and NSE activity (figures 1 and 2).

The cirrus sac and testes of the male reproductive system, and vagina of the female reproductive system showed AChE activity (figures 3 and 5). The cirrus and embryos also showed NSE activity (figures 5 and 6). Enzyme assay indicated the presence of specific AChE since acetylcholine was hydrolysed at a higher rate than butyrylcholine. *In vitro* studies showed that some enzyme was also secreted.

The results show that AChE activity is present mainly in the nervous system of this cestode. When the nervous system of several species of cestodes[10,11], was incubated in *O*-acetyl-5-bromoindoxyl for localization of NSE, AChE activity was also found. In this study, the absence of reaction when eserine (10^{-5} μM) was used as an inhibitor with AThChI indicates that AChE is the chief enzyme. Contradictory results have been obtained in studies on the occurrence of AChE in embryos of cestodes[2,3,12,13]. But Rybicka[14] reported that only true AChE activity occurs in embryos. In the present study, the embryos showed only NSE activity.

AChE present in suckers and rostellum may be the chief neurotransmitter in the neuromuscular processes in contraction and eversion. In addition to neuromuscular transmission other roles have also been ascribed to this enzyme. Schwabe *et al.*[15] suggested that AChE is involved in permeability and osmoregulation of the hydatid cyst wall of *Echinococcus granulosus*. Arme[1] observed involvement of the enzyme in lipid excretion in *Ligula intestinalis*. According to Lee[10] and Ogilvie *et al.*[16] this enzyme acts as a biochemical hold-fast.

Parasite secretions may elicit immune response in the host. In the case of *A. multitesticulata* also, the secreted AChE may induce an immune response.

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FUNCTIONAL SIGNIFICANCE OF ARGINASE IN AESTIVATING SNAIL, *PILA GLOBOSA* (SWAINSON): NEUROENDOCRINE INVOLVEMENT

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THE mechanisms involved in the regulation of aestivation metabolism in molluscs have not been worked out thoroughly. This communication reports the functional significance of arginase in the hepatopancreas of the aestivating snail (*Pila globosa*) and possible neuroendocrine involvement in the regulation of aestivation metabolism with reference to excretion.

The collection, maintenance and mode of induction of aestivation in snails have been described elsewhere[1]. Three-month-aestivated snails were selected for the study. The five ganglia, viz. cerebral, buccal, pleuropedal, supra-intestinal and visceral, were isolated separately from both normal and aestivated snails. Ganglionic extracts (1%) were prepared in 80% ethanol and centrifuged at 1000 *g* for 20 min. The extracts (0.2 ml) prepared from active snails were injected into five batches (six snails each) of aestivating snails through a hole drilled near the operculum under aseptic conditions and the holes were closed immediately with sealing wax. Similarly, extracts of ganglia from aestivated snails were administered to five batches (six snails each) of active snails. Control snails received only 0.2 ml of ethanol. The snails were allowed to move freely in their containers in autoclaved water containing 1000 IU of penicillin per litre. After 60 min the hepatopancreas was isolated in the cold for estimation of

Snail

Table 1 *Effect of administration of ganglionic extracts on specific activity of arginase (μmol urea/mg protein/h) and urea (μmol/g wet wt) in hepatopancreas of freshwater snail Pila globosa*

	Control	Cerebral	Buccal	Pleuropedal	Supra-intestinal	Visceral
Active snails administered ganglionic extracts from aestivated snails						
Arginase	0.032 ± 0.002	0.015 ± 0.001 (−53.13%)	0.011 ± 0.001 (−65.63%)	0.024 ± 0.002 (−25.00%)	0.01 ± 0.0003 (−68.75%)	0.009 ± 0.0004 (−71.88%)
Urea	0.52 ± 0.046	0.25 ± 0.013 (−51.92%)	0.16 ± 0.001 (−69.23%)	0.39 ± 0.06 (−25.00%)	0.11 ± 0.008 (−78.85%)	0.13 ± 0.009 (−75.00%)
Aestivating snails administered ganglionic extracts from active snails						
Arginase	0.052 ± 0.004	0.035 ± 0.002 (−32.69%)	0.030 ± 0.002 (−42.31%)	0.045 ± 0.003 (−13.46%)	0.026 ± 0.001 (−50.00%)	0.021 ± 0.001 (−59.62%)
Urea	0.96 ± 0.071	0.52 ± 0.032 (−45.83%)	0.36 ± 0.025 (−62.50%)	0.76 ± 0.043 (−20.83%)	0.25 ± 0.021 (−73.96%)	0.29 ± 0.024 (−69.79%)

Each value is mean ± SD of six individual observations.
All values are significant at $P < 0.05$.

arginase[2] and urea[3]. Protein was estimated by the method of Lowry *et al.*[4]

The specific activity of arginase and level of urea in hepatopancreas of normal, aestivated and ganglionic extract-administered snails are presented in table 1. The results show that during aestivation the specific activity of arginase increased by 62% and was accompanied by an increase in urea. However, when ganglionic extracts were administered to the snails (extracts from active snails to aestivated snails and *vice versa*), arginase activity and urea level decreased. Visceral ganglionic extract produced the greatest effect on arginase and supra-intestinal ganglion extract produced the greatest effect on urea. In general, the effect produced by ganglionic extracts from aestivated snails was more than that of extracts from active snails.

The increased arginase activity and urea in the hepatopancreas of aestivating snail are due to increased availability of arginine through elevated proteolysis[5]. This elevated arginase activity and urea reflect the metabolic centrality of hepatopancreas in combating ammonia stress during aestivation. When ganglionic extracts of aestivated snails were administered to active snails, both arginase activity and urea level decreased. It appears that endocrine factor(s) in ganglionic extracts caused an increase in urease activity, which in turn enhanced the hydrolysis of urea, resulting in the decreased level of urea. In aestivating snails, ganglionic extracts from active snails had a similar effect, causing reduced formation of urea. The variation in the extent of reduction in arginase activity and urea produced by the different ganglionic extracts indicate the presence of more than one humoral agent in the nerve ganglia.

Prima facie, the altered arginase activity pattern indicates the differential ureogenic capacity of the tissue. Variation in the urea level is an index of the balance between rates of formation and mobilization. The 'high' and 'low' activity of arginase in the hepatopancreas of aestivating and active snails also exemplify such 'balance'. However, low ureogenic capacity as reflected by decreased arginase activity and low urea content in the hepatopancreas of snails administered ganglionic extracts indicates that hepatopancreas may not be an 'important contributor' to urea production.

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EFFECT OF pH ON PHOSPHATE SOLUBILIZATION BY MICROBES

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THE pH of the nutrient medium is one of the important factors determining the uptake of inorganic nutrients by plants. The concentration of hydrogen and hydroxyl ions exerts a definite effect on the vital activity of soil microorganisms and also on phosphorus availability[1]. The pH of the soil is 5.6–6.2 in sandy soils and 6.0–6.8 in mineral soils[2]. The performance of phosphate-solubilizing microorganisms (PSM) may also be affected if the pH of the nutrient medium is altered. The present investigation was undertaken to evaluate the behaviour of PSM at different pH values.

Fifty ml of Pikovskaya's[3] broth was dispensed in 100 ml conical flasks. Tricalcium phosphate (an insoluble inorganic source of phosphate) equivalent to 25 mg P_2O_5 was added separately to each flask. Four sets were prepared, for pH values of 5.4, 6.5, 8.0 and 9.0. The medium was sterilized at 10 psi for 30 min and pH was also recorded after sterilization. The medium was inoculated with 0.5 ml suspension of 4-day-old *Pseudomonas striata*, *Bacillus polymyxa*, *B. circulans*, *B. subtilis* and *Aspergillus niger* cultures (each in triplicate) in Pikovskaya's broth. Uninoculated controls were maintained. Phosphorus was estimated by the chloromolybdoblue colour[4] method at 660 nm using Hilger's absorptiometer after 3, 6, 9, 15 and 21 days of incubation at 35°C. Change in pH of filtered medium was also recorded. The sterilization resulted in reduction of the original pH of the medium (from 9.0 to 8.0, 8.0 to 7.0, 6.5 to 6.0 and 5.4 to 5.4), and this is taken as initial pH.

Phosphate solubilization data (figure 1) show that different isolates had maximum dissolution activity at different values of pH. Both incubation period and pH of the nutrient solution were found to influence the performance of PSM.

At pH 7.0 and 8.0, both *P. striata* and *B. polymyxa* showed a gradual increase in phosphate solubilization up to day 9 of incubation followed by a steady decrease. At pH 5.4 and 6.0, however, the increase in phosphate solubilization was recorded up to day 15. Although there was not much variation in the performance of *P. striata* the optimum pH for both these isolates was 6.0.

B circulans showed increase in phosphate solubilization up to day 15 at pH 5.4, 6.0 and 8.0; at pH 7.0 a

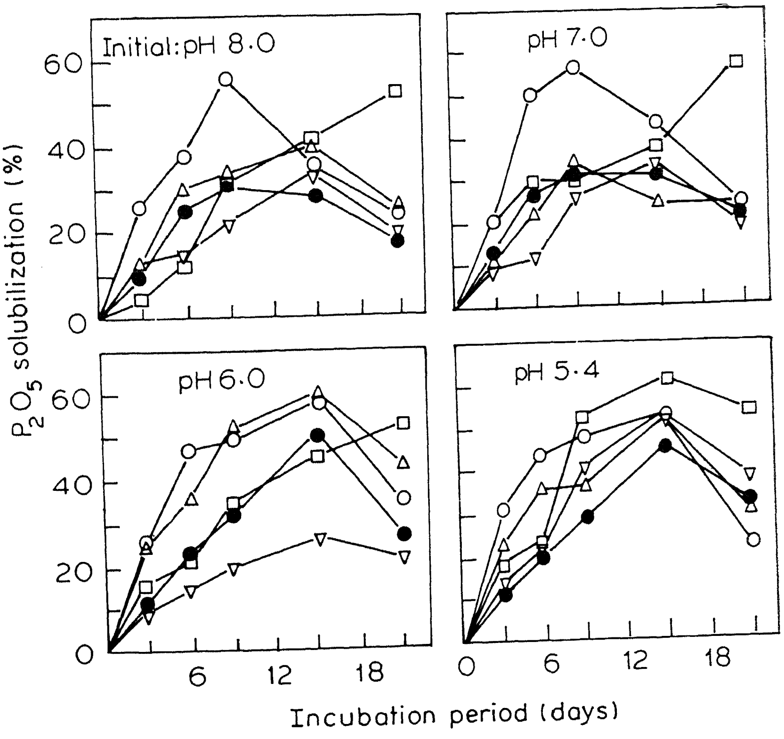

Figure 1. Phosphate solubilization by *P. striata* (○), *B. polymyxa* (●), *B. circulans* (△), *B. subtilis* (▽) and *A. niger* (□) at different pH values.

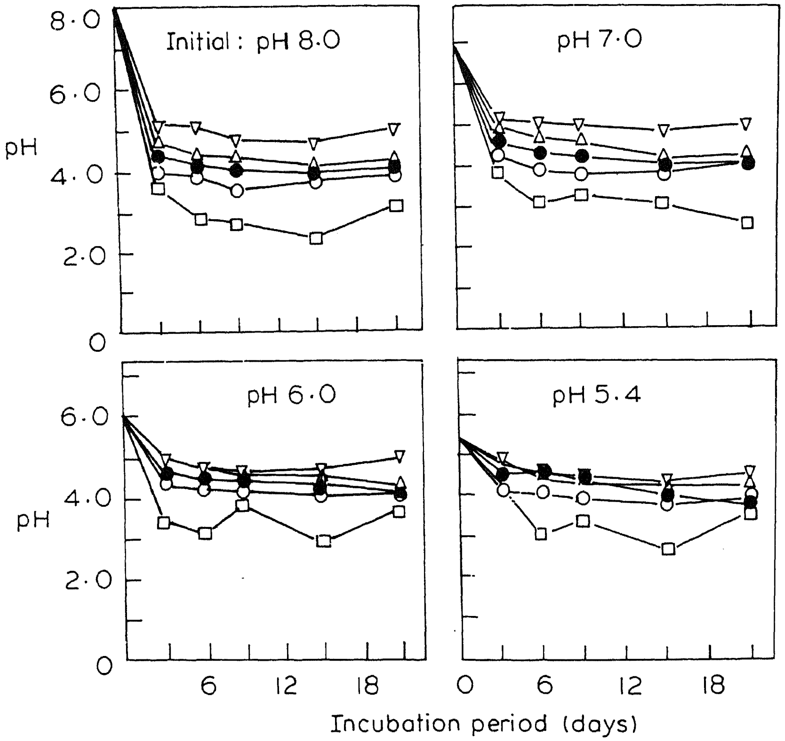

Figure 2. Change in pH of growth medium containing tricalcium phosphate during growth and phosphate solubilization activity of *P. striata* (○), *B. polymyxa* (●), *B. circulans* (△), *B. subtilis* (▽) and *A. niger* (□).

decrease was observed after day 9. The pH optimum for *B. circulans* was also 6.0, where the highest dissolution of 60.32% was recorded.

In the case of *B. subtilis* there was an increase in phosphate solubilization up to day 15 at all four pH values, followed by a decrease. However, pH 5.4 was optimum for activity. Thimann[5] reported the growth of *B. subtilis* within the pH range 4.5–8.5, which supports our findings.

At pH 5.4, the fungus *A. niger* was found to have better solubilization activity than the bacteria *P. striata*, *B. polymyxa*, *B. circulans* and *B. subtilis*. Fungi are known to grow and perform better under medium-acidic condition[6,7].

The drop in solubilization after a maximum value may be attributed to deficiency of nutrients in the medium after an optimum period of incubation[8,9].

The change in pH of filtered medium (figure 2) was almost in the same range for all four initial values. Drop in pH was highest in the medium inoculated with *A. niger* and least in medium inoculated with *B. subtilis*. The fall in pH clearly indicates the production of acids, which is considered to be the sole mechanism responsible for the solubilization of insoluble phosphates. There was not much difference in the amount of tricalcium phosphate solubilized between the four organisms.

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HETEROCHROMATIN OF RYE AND TRITICALE—REPETITIVE SEQUENCES

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BANDING techniques have provided valuable information about organizational aspects of chromosomal heterochromatin in rye, wheat and triticale. The chromosomes of the three species show clear structural differences. Rye chromosomes have large blocks of heterochromatin located at the telomeric ends (identifiable by C-banding, figure 1), while in wheat, heterochromatin is present in intercalary positions.

The molecular structure of heterochromatin in rye and wheat has been studied[1-3]. The studies revealed that the DNA density in heterochromatic regions[4] is 1.5 to 4 times[5] higher than that in euchromatic regions. Heterochromatin composed of late-replicating repetitive DNA sequences[6] has a direct relationship with it[7]. The proportions of single-copy and repetitive DNA in wheat, rye and triticale are presented in table 1. Kinetic analysis (renaturation of denatured DNA) of genome organization in rye and wheat has revealed that repeated sequences are interspersed with unique sequences. The repetitive sequences of heterochromatin of rye and wheat are classified into three subfractions: (i) low repeat (consisting of about ten copies), (ii) intermediate repeat (a hundred to a few thousand copies), and (iii) high repeat (over a million copies). It has also been demonstrated that most of the highly repeated sequences represent constitutive heterochromatin[8,9] and are species-specific[10,11].

The repetitive DNA makes up about 50% of the total DNA in triticale; the highly repeated fraction contains two families, of 3×10^7 and 5×10^6 repeats, which constitute 8.3 and 2.4% of the genome. In rye the repetitive DNA makes up 75–90% of the total DNA and is grouped into four repetitive DNA fami-

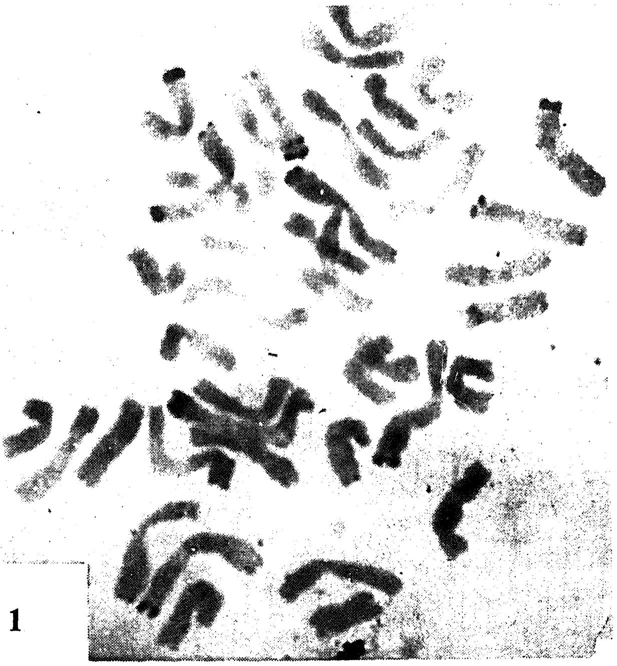
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Figure 1. C-banding of rye chromosomes, showing heterochromatin at the telomeric ends.

Table 1 *Repetitive sequence organization pattern in wheat, rye and triticale*

Sequence	Wheat	Rye	Triticale
Short non-repeats (single-copy sequences) interspersed with short repeated sequences (%)	30–40	25–35	45–55
Interspersed repeats or tandem repeats (%)	60–75	65–75	45–55
Long non-repeats (%)	2	5	7

lies[6,12]. Two repetitive families were confined to telomeric heterochromatin identifiable by Giemsa C-banding, while the remaining two are present in constitutive heterochromatin. Of these four, three sequences were rye-specific (480 base pairs, 610 bp and 630 bp) and one (120 bp) was common to rye and wheat genomes[7]. All the four families have been identified and studied in different species related to rye (*Secale cereale* (rye), *S. montanum*, *S. vavilovii*, *S. silvestre*, *S. africanam* and *S. iranicum*). Each species was unique with respect to the chromosomal distribution and amount of the repeated sequence families. Species other than *S. cereale* have a lower percentage of repeated sequences and heterochromatin. In most of the families the repeated sequences are similar, as indicated by restriction analysis using *Eco*RI, *Hin*dIII and *Hae*III. As many as 12 different DNA sequences specific to rye chromosomes have been characterized and described[13]. *In situ* hybridization with a cDNA probe demonstrated that the highly repetitive DNA sequence common to both rye and wheat genomes is distributed either on B chromosomes or on 4A and 7A (ref. 14) chromosomes. Recently, Metzlaff *et al.*[15] discovered wheat-specific repetitive DNA sequences (pTa 1, pTa 2, pTa 7, pTa 8) that are GC-rich, as against rye repetitive sequences that are AT-rich[9]. In triticale these sequences were shown to affect chromosome movement[16] and pairing[8], which consequently influence meiotic stability. Selective deletion of some of the repetitive sequence families specific to rye has been shown to improve meiotic stability in the triticale cultivar Rosner[7], thereby indicating that the heterochromatin of rye chromosomes in triticale does undergo alterations.

A study of DNA and heterochromatin content within the genus *Secale* indicated that the amount of heterochromatin increased during evolution[4]. Flavell *et al.*[17] showed that the magnitude of the DNA/heterochromatin differences in the repeated sequences of various species is related to the extent of evolutionary divergence between the species. Further, the amount of telomeric heterochromatin in *S. cereale* varies between different populations, between different individuals within a population and even between different cells in a single root-tip[18]. It was hypothesized that these variations in repetitive DNA originate by the sudden disproportionate replication (saltatory replication) of specific nucleotide sequences in heterochromatin. These were regarded as the major event in the evolution of a species. Variation in these sequences occurs by mutations, deletions, translocations and rearrangements[19]. Chromosomal DNA content is therefore maintained by a balance between sequence amplification and deletion (termed 'turning over')[18] of the genome.

Unequal chromatid exchanges are believed to have been a source of quantitative change in an array of repeated DNA sequences during evolution[20]. Unequal meiotic exchange within the repeated sequences near the euchromatin–heterochromatin junction may be a source of gradual quantitative variation in the repeated sequences[21]. The B chromosomes present in many species of rye have been considered to be another source of variation in the heterochromatin of normal chromosomes, through translocation events, incorporating segments from the B chromosomes. Special cases such as transposable elements, gene conversion and aberrations in mitotic divisions[22] are some of the rare events that are also responsible for changes in the genome.

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MULTIPLE FORMS OF HUMAN URINARY γ-GLUTAMYLTRANSPEPTIDASE

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OF several enzymes excreted in human urine, γ-glutamyltranspeptidase (GGTP) is considered as one of the important enzyme markers that can be used to assess the uro-renal system. Among different body fluids, urine was found to be a rich source of the transpeptidase. In cancer patients, though urinary GGTP activity was found to be increased, there was no significant change in serum transpeptidase activity. The work described here is part of a study of transpeptidase isozymes in urine of normal individuals and cancer patients.

Urine samples collected from normal and diseased individuals were dialysed extensively against 10 mM Tris–HCl buffer, pH 8.0, for about 24 h. The dialysed urine samples were centrifuged at 3,500 rpm for 15 min, and supernatants were freeze-dried for chromatographic and electrophoretic experiments.

A known aliquot of a solution of desalted concentrated urine samples in 10 mM Tris–HCl buffer, pH 8.0, was passed through a DEAE-cellulose column pre-equilibrated with the sample buffer. The column was developed with the sample loading buffer. The unbound protein fraction contained transpeptidase activity, and was called unbound isozyme. After washing the column with 2–3 bed volumes of the sample buffer, bound transpeptidase activity was desorbed into two peaks, bound-I and bound-II, by applying a linear salt gradient (0 to 0.5 M NaCl) in the buffer. The enzymatically active fractions were dialysed and concentrated for polyacrylamide disc gel electrophoresis (PAGE)[1]. The concentrated samples were suspended in 1% Triton X-100 and electrophoresed in 7.5% acrylamide gels containing 0.01% Triton X-100. After the electrophoresis, transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2]. Each of the three enzymatically active fractions yielded one activity band.

Gel filtration chromatography of the desalted freeze-dried samples through Sephadex G-200 gave two enzymatically active peaks. Electrophoretic (PAGE) analysis of the first enzymatically active peak (high mol. wt form) showed one transpeptidase isozyme while that of the second (low mol. wt form(s)) peak gave two enzymatically active bands.

Direct electrophoresis of the desalted concentrated urine samples in 1% Triton X-100 gave three enzymatically active bands in the zymogram (figure 1). The pattern of transpeptidase isozyme distribution in the electrophoretogram was observed to be comparable to that of detergent-solubilized human kidney transpeptidase[3] preparation.

Rambabu *et al.*[4] showed the existence of the two transpeptidase isozymes and that the transpeptidase activity is increased in urine of leukaemia patients. But we have detected three transpeptidase isozymes in human urine. Grossly speaking, we also observed an increased level of the total transpeptidase activity in urine of leukaemia patients, but detailed investigations on several leukaemia patients have indicated that the activity of the unbound transpeptidase

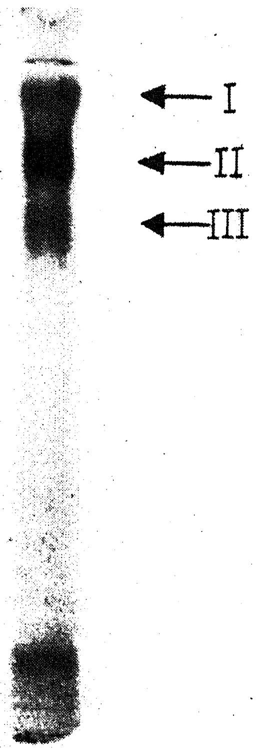

Figure 1. Polyacrylamide disc gel electrophoresis pattern of dialysed normal human urine. About 150 μg protein was electrophoresed and transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2].

isozyme is lower and that of the bound isozyme forms considerably higher to give an elevated total urinary GGTP activity in leukaemia patients[5]. However, other cancer patients (except those under treatment) showed no change in urinary transpeptidase activity.

Tsuji *et al.*[6] have reported that liver GGTP presumably leaks into serum, because many properties of liver and serum GGTP are comparable. Hence the level of GGTP in the serum can be used as an index of hepatic function.

GGTP in the human kidney is located on the extrinsic surface of the brush border membrane and, to some extent, elsewhere in the kidney[7]. Although the molecular weights of GGTP from urine and kidney were found to be different[8], Scherberich *et al.*[9] have shown immunological identity between urinary GGTP and GGTP of proximal tubule cells. Based on these similarities (immunological and electrophoretic properties), it can be presumed that urinary GGTP is leaked from the kidney membranes into urine, and that any change in the proportions of the urinary transpeptidase isozymes can be used to assess the dynamic status of the brush border membrane. Further, this type of study might be of diagnostic importance in differentiating between non-renal and renal conditions. However, more studies are required to establish the actual clinical use of estimating the isozymes of urinary enzymes like GGTP, alanine aminopeptidase, alkaline phosphatase, lysozyme, etc.

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INDUCTION OF LYSOSOMAL ENZYMES IN EHRLICH ASCITES TUMOUR CELLS BY BROMOPHOS

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In mammals xenobiotics are detoxified mainly in liver cells by enzymes located predominantly in the endoplasmic reticulum. These enzymes are known as

Enzyme induction; Bromopho

'microsomal detoxification enzymes'[1]. They have been studied extensively in safety evaluation of drugs and environmental chemicals. However, very little is known about the role of lysosomal enzymes in the detoxification of xenobiotics. Although lysosomes have been implicated in the metabolism of xenobiotics, a direct demonstration of the involvement of lysosomal enzymes has not been made. Hence the induction of lysosomal enzymes in cultured Ehrlich ascites tumour (EAT) cells by bromophos, a non-systemic organophosphorus insecticide, was studied.

EAT cells were cultured in the peritoneal cavity of Swiss mice and harvested 10–12 days after transplantation. The aspirated cells were suspended in saline. Contaminating erythrocytes, if any, were lysed by a brief hypotonic shock. Viability of the cells was determined by trypan blue dye exclusion[2]. Cells (5×10^6/ml) were then suspended in saline and incubated without bromophos or with 50 or 100 nmol of bromophos (*O,O*-dimethyl-*O*-(2,5-dichloro-4-bromophenyl)-phosphorothioate) at 37°C for 1 h. After the incubation the cells were pelleted by centrifugation at 800 *g* for 5 min. The cell pellet was homogenized in a Potter–Elvehjem homogenizer in saline containing 0.01% Triton X-100. The cell homogenate and cell supernatant were used for enzyme assays. Hexosaminidase (EC 3.2.1.52) was assayed using 4-methylumbelliferyl-*N*-acetyl-*β*-D-glucosaminide as substrate[3]. *β*-Glucuronidase (EC 3.2.1.31) was assayed using phenolphthalein glucuronide as the substrate[4]. Aryl ester hydrolase (EC 3.1.1.2) was assayed using *α*-naphthyl acetate as substrate[5]. Glucose-6-phosphatase was assayed by measuring the inorganic phosphate[6] released from glucose-6-phosphate[7]. Protein was determined by Lowry's method[8].

More than 90% of freshly harvested cells were viable, by trypan blue dye exclusion. Activities of the four enzymes assayed in cell pellet and cell supernatant are shown in figure 1. In the cell pellet activities of hexosaminidase, glucuronidase and aryl ester hydrolase increased with increasing concentration of bromophos. In the cell supernatant, activity of hexosaminidase increased with increasing concentration of bromophos, whereas increase in aryl ester hydrolase activity was marginal and glucuronidase activity did not increase. Glusose-6-phosphatase activity in both cell pellet and the cell supernatant decreased with increasing concentration of bromophos.

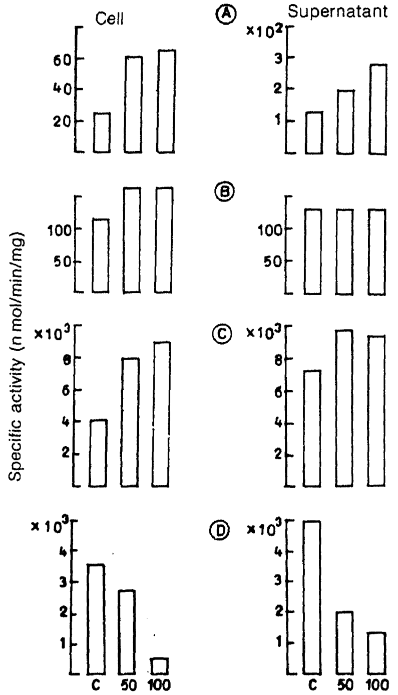

Figure 1. Effect of bromophos (C, control; 50, 50 nmol; 100, 100 nmol bromophos) on activities of (A) hexosaminidase, (B) glucuronidase, (C) aryl ester hydrolase and (D) glucose-6-phosphatase in EAT cells and cell supernatants. Each result is the mean of two independent determinations.

The study of induction of enzymes involved in xenobiotic metabolism in experimental animals has many inherent problems. First of all the distribution of xenobiotic in the different tissues must be known. Secondly the response of different tissues to the same xenobiotic may be different owing to factors such as hormonal interactions and immunological responses. Hence in our study we used cultured EAT cells since they provide a homogeneous population of rapidly dividing cells. Our results show that bromophos stimulates the activity of lysosomal enzymes and that at least some of these enzymes are released into the medium. Glucose-6-phosphatase, an enzyme located primarily in the endoplasmic reticulum, is not normally induced by xenobiotics. Hepatotoxic chemicals have been shown to decrease its activity in liver microsomes[9]. Xenobiotics like phenobarbital have been shown to mask the activity of the enzyme by inducing a change in the endoplasmic reticulum[10]. In our study, although the cause of inhibition of glucose-6-phosphatase is not known, bromophos, or some metabolic product of bromophos, acting as an inhibitor of the enzyme cannot be ruled out. Such inhibition by organophosphorus compounds is well known. Although the significance of the increase of lysosomal enzymes on the one hand and the selective release of some of them on the other in EAT cells is not known, lysosomal enzymes may be important in xenobiotic detoxification reactions.

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5 December 1988

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TRACE METALS IN CANCER TISSUES

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CURIOSITY about the trace metal composition of cancer tissues was aroused by the accumulation of copper and certain other trace elements that one of us (TRD) had observed in the affected skin of cases of leprosy, leucoderma and eczema some time ago (unpublished). Reference to the literature gave very little information on the mineral profiles of cancerous tissues although several metals are implicated in carcinogenesis and allergy in humans and experimental animals[1]. We have analysed a number of cancerous and the corresponding normal tissues, procured through the courtesy of local hospitals, for certain physiologically important trace elements—Ca, Cd, Co, Cu, Fe, Mg, Mn and Zn. The results are presented in this communication.

The analyses have been completed in six cancer cases and an equal number of normals. In the cancer patients diagnosis had been confirmed on the basis of clinical and histological criteria. These include two cases each of cervical, oesophageal and gastric cancer. The normal cases were adjudged to be so on the basis of freedom from all overt clinical signs and symptoms of cancer or other diseases and the normal histological picture of the biopsy specimens of the specific tissues taken for analysis.

The tissues were excised by biopsy guided by endoscopy, parallel specimens being taken from the same area for histological examination. Tissues were preserved in absolute alcohol until analysis. They were dried, digested in a mixture of sulphuric and nitric acids[2], and made up to volume, and aliquots taken for estimation of the various mineral elements by atomic absorption spectrometry[2] (Perkin–Elmer Model 403 instrument). The same wet-digestion and subsequent analytical steps were followed with blood sera also.

The data are presented in table 1. The affected tissues in three cases of cancer of the colon and two cases of rectal cancer were also analysed, but as the corresponding normal tissues were not available, the data are not included here. There were no significant differences in the pattern of minerals assayed in blood sera between nine normal and six cancer cases and so they are also not reported here.

In spite of the wide individual variations, the trace metal spectra of the cancerous tissues examined compared with those of the corresponding norma tissues (table 1), reveal several interesting features The general trend of the results indicates that, in an given type of cancerous tissue, the sense of departur from the normal is the same for all the metal examined. Both cancerous cervix and cancerou oesophagus show decreases ranging from 25 t 80%, reckoned on the basis of the mean values. O the other hand, in cancerous gastric mucosa th concentration of the metals increases by two t seven times. That the former two tissues are ecto dermal and the latter endodermal in origin may be

Table 1 *Trace metal profiles of some cancer and the corresponding normal tissues*

Tissue		Trace metal (μg/g tissue) Ca	Cd	Co	Cu	Fe	Mg	Mn	Zn
Cervix	N1	530	17	61	33	190	568	15	91
	N2	1193	23	91	30	284	1136	23	634
	C1	146	4	16	13	83	276	4	80
	C2	285	16	43	17	100	446	7	185
Oesophagus:	N3	1437	100	500	233	687	1500	125	1000
	N4	2500	150	500	412	100	3125	125	1437
	C3	1028	65	212	58	718	1283	40	514
	C4	900	78	216	85	519	1028	31	700
Gastric mucosa:	N5	1380	38	108	92	448	1708	20	341
	N6	1120	71	142	100	552	1317	22	619
	C5	6375	250	750	525	2000	7500	125	337
	C6	13500	150	300	200	750	7750	100	3000

N, Normal; C, cancerous.

determining factor in this differential response to the oncogenic process. In both types, however, the maximum effect appears to be on calcium and manganese, followed by magnesium in the gastric mucosa, zinc in the cervix and cobalt in the oesophagus.

The absence of any significant change in the pattern of these minerals in serum indicates that the changes observed are localized to tissues and do not involve any disturbances in the general metabolism of the minerals.

The data appear to be interesting as first results, although the number of tissues of each type analysed is small to draw firm conclusions. They show the importance of minerals in oncology and underline the need for further extension of the analyses to a larger number and variety of tissues. These studies are in progress.

The authors thank Dr Rama Rao, St. John's Medical College and Hospital, Bangalore, and Dr Susheelamma of the Corporation Hospital, Vijayanagara, Bangalore, for providing authentic samples of tissues and sera. Thanks are also due to the Director, Geological Survey of India, Bangalore, for atomic absorption spectrometry facilities and to Mr M. V. L. Rao, Mysore, for his encouragement and helpful criticism.

15 June 1988; Revised 16 February 1989

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EFFECT OF POTASSIUM EMBELATE, AN ANALGESIC COMPOUND, ON BRAIN MONOAMINE UPTAKE

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It has been suggested that noradrenaline (NA) and 5-hydroxytryptamine (5-HT) play a major role in the transmission of nociceptive impulses in the brain. However, the opioid analgesics and antagonists are reported to interfere with NA and 5-HT inactivation by blockade of neuronal uptake[1-3]. The potassium salt of embelin (from *Embelia ribes* Burm.) is a new compound which has shown analgesic activity when administered orally to laboratory animals[4]. It was found to be centrally active but did not produce narcosis or show addictive property of morphine[4]. The present study was undertaken to assess any possible interaction of this drug with the uptake of monoamine transmitters in rat brain synaptosomes.

Male Charles–Foster rats (150–175 g), housed at constant temperature (28 ± 2°C) with free access to water and food, were divided into two groups of 12 animals each. One group of animals was administered potassium embelate orally (20 mg/kg body weight). The control group was given vehicle only, which consisted of distilled water and Tween-80. Thirty min after drug administration, animals were killed by decapitation, and the brains quickly removed and homogenized in ice-cold Krebs–Ringer buffer using a Teflon-fitted all-glass homogenizer. Synaptosomes were obtained as described by Gray

Table 1 *Uptake of 5-hydroxytryptamine and noradrenaline by brain synaptosomes of untreated and potassium embelate-treated rats*

Group	Incubation time (min)	5-HT (dpm/mg protein/ 15 min)	Per cent uptake	NA (dpm/mg protein/ 15 min)	Per cent uptake
Control	0	18840 ± 1122	43.3	7570 ± 1117	49.0
	15	44050 ± 3092		21310 ± 3533	
Treated	0	19040 ± 909	51.6	5090 ± 989	53.4
	15	50010 ± 3252		20040 ± 3572	

$$\text{Per cent uptake} = \frac{\text{counts after 15 min incubation} - \text{counts at zero time}}{\text{initial counts added per assay}} \times 100.$$

Control vs treated non-significant (*t* test).

and Whittaker[5]. The crude fraction (P_2) was diluted with Krebs–Ringer buffer containing 15 μM iproniazid to give a final concentration of 1 mg/ml of protein. Protein was determined by the method of Lowry *et al.*[6] Samples (0.5 ml) were pre-incubated at 30°C in a water bath. After 5 min 60,000 dpm/assay tube of $[^{14}C]$5-HT or 28,000 dpm/assay tube of $[^{3}H]$NA was added. Another set of identical samples was incubated at 0°C to serve as control. After 15 min of incubation, the reaction was stopped by adding 0.5 ml of ice-cold buffer and the samples rapidly filtered through Whatman GF/B filters. The radioactivity was measured in a Beckman liquid scintillation counter (LS 3150). The difference between the $[^{14}C]$5-HT and $[^{3}H]$NA counts at 30 and 0°C was taken as a measure of uptake. All experiments were done in duplicate.

The potassium salt of embelin was prepared according to procedures standardized in this laboratory. 5-[2-^{14}C] Hydroxytryptamine binoxalate (specific activity 56.3 mCi/mmol) and DL-[7-^{3}H (N)] norepinephrine (noradrenaline) hydrochloride (specific activity 13.8 Ci/mmol) were obtained from New England Nuclear, USA. Iproniazid phosphate was purchased from Sigma Chemical Co., USA.

It was shown earlier[4] that the analgesic activity of potassium embelate increased in the early period (0–15 min) and reached a maximum at 30–60 min after drug treatment (20 mg/kg body weight). The effect of this drug on monoamine uptake was studied during peak analgesia, 30 min after drug administration. The results (table 1) showed that there was no significant change in either 5-HT or NA uptake. This draws attention to the fact that since potassium embelate did not interfere with the neuronal uptake of 5-HT and NA, the inactivation of monoamines would also be unaffected. Cosequently, the possibility of accumulation of cerebral monoamines to a toxic level could be precluded in potassium embelate-treated animals. In this regard, it may be mentioned that opioid analgesics impair the uptake and inactivation of 5–HT and NA in brain synaptosomes[7–9], which may cause enhanced pressor response to endogenous and exogenous monoamines. The present results however suggest that such a clinical complication may not exist with the use of potassium embelate.

3 November 1988

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BOOK REVIEWS

Annual Review of Pharmacology and Toxicology, vol. 29, 1989, pp. 543, (eds) Robert George and Ronald Okun (Published by Annual Reviews Inc, 4139 El Camino way, Palo Alto, California 94303–0897, USA) Price: USA $34; Elsewhere $38.

Annual Reviews has been doing a signal service to students of pharmacology and toxicology by highlighting, in some of the recent volumes, the achievements of pioneers in the field and the home and academic environment in which they worked. The volume under review carries a tribute to Bernard B. Brodie, the father of biochemical pharmacology. The period 1950–1970 witnessed an amazing array of discoveries in the Laboratory of Chemical Pharmacology of the National Heart Institute. Differential extraction of drugs and their metabolites by solvents of differing polarity was the principle Brodie used in perfecting analytical methods for drugs. This approach has contributed significantly to the speedy approval and release by regulatory agencies of drugs for human use. Another fundamental discovery was the role of mixed function oxidases or drug metabolizing enzymes, which are now of significance not only for drugs but all xenobiotics, including environmental pollutants. The studies conducted under Brodie's direction on the relationship between 5-hydroxy-tryptamine (serotonin) and reserpine led to the development of the new discipline of neurochemical pharmacology. The outcome was the uncovering of the role of monoamine oxidases and the discovery of drugs used extensively today in the treatment of depression, hypertension and cardiovascular ailments.

Granules located in the atria contain three important peptides. These peptides are released into circulation in a number of ways. The mechanism of atriopeptin regulation and function and atriopeptin receptors have been reviewed. Percutaneous absorption of drugs is critical for the efficacy of drugs applied to live human skin. Glucocorticosteroids play a key role in the release of drugs applied on the skin and transported by vehicles. There is much more to learn about penetration enhancers. Stratum corneum not only acts as a barrier but also as a reservoir of the drugs.

Inositol phosphates and 1,2-diacylglycerol are formed when membrane-bound phosphoinositides are hydrolysed in response to receptor stimulation. Among the selective receptors located on the cell surface are those with which neurotransmitters, neuromodulators, hormone and growth-promoting factors interact to elicit their diverse physiological and metabolic responses. The transduction and amplification of extracellular signals are mediated by the surface receptors by generating inositol phosphate and 1,2-diacylglycerol, which function now as second messengers. DNA synthesis, cellular metabolism, growth, synaptic transmission, neuronal membrane excitability, sensory transduction, muscle contraction, motility and platelet aggregation are some of the physiological processes in which the 'dual messenger' system operates. Recent advances in the knowledge of CNS and related neural tissue receptors and phosphoinositide hydrolysis are reviewed by De-Maw Chaung.

Technical advances in the area of X-ray crystallography of macromolecules have made rational antiviral drug design a reality today. Azidothymidine, an effective agent against human immunodeficiency virus (HIV), the aetiologic agent of AIDS, is now readily available in the market. Other commercially exploited antiviral agents—acylclovir, iodoxuridine, amantidine and others—were all designed on an understanding of a prototype drug/viral structure or functional protein interaction at the atomic level. All these share the important property of the ability to inhibit the process of uncoating (following adsorption to and penetration of the cell membrane) by which viral nucleic acid is released from the viral protein shell or capsid into the cytoplasm of the infected cell. These interesting developments have been reviewed by McKinlay and Rossmann.

Leukotrienes derived from arachidonic acid have been shown to possess a constellation of pharmacologic effects on respiratory, cardiovascular and gastrointestinal systems. Receptor antagonists of leukotriene are potential therapeutic agents. Two reviews discuss the recent developments in this area and the nature of the receptors, which have now been found in platelets as well as in many vascular tissues.

A review of the biochemical mechanisms of hepatic peroxisome proliferation by xenobiotics, of interest to those working in the area of cancer induction, is presented by Lock *et al.* For those concerned with basic molecular mechanisms of action of toxic chemicals, the review by Juchan on the bioactivation of teratogens, the critical account given by William of the methods for evaluating chemical genotoxicity, and the one on human cytochrome P-450 by Guengerich will be of considerable interest.

The exciting developments anticipated in the chemical coding of neurons and the involvement of release and interaction of several transmitter substances are presented by Furnen *et al.* Thrombolytic therapy in myocardial infarction has been legitimized by the approval of the use of streptokinase and intravenous recombinant tissue plasminogen activator. The fundamental biochemical advances made in this area are reviewed by Bang *et al.* Other chapters deal with amino acid-based neurotransmitters, the recent advances in unravelling the role of 'transfer factor' involved in the transfer of immunity in tissue transplants, development of animal models for reproducing human stroke, and the nature and magnitude of indoor pollutants in highly developed societies. The review of reviews on recent advances in the pharmacology of drug addiction is very topical. Considering the rate at which progress is being made in biochemical pharmacology and toxicology, an area of great relevance to human health, one wonders whether *Annual Review of Pharmacology and Toxicology* may have to be replaced by half-yearly reviews or critical reviews on select topics.

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Space-Related Materials Advances in Materials Technology: Monitor, (Published by Dept for Industrial Promotion Consultations and Technology, UNIDO, P.O. Box 300, A 1400, Vienna, Austria), pp. 64.

This UNIDO publication, a part of its 'Advances in Materials Technology: Monitor', provides an excellent overview of developments in both the use of materials for space applications and in the processing of materials in space.

The theme for the entire publication is set in the first article by Mir Akbar Ali. Ali reviews work on space materials processing in the USSR, USA and Europe. Through the illustrative example of the growth of silicon ribbon for integrated circuit fabrication in the zero-*g* condition of space the advantages that can accrue from the use of space processing is highlighted. While it is difficult to draw firm conclusions on the economics of manufacture of different materials in view of the paucity of published data, the methodology is useful in assessing potential products for space manufacture.

The second part of Ali's article focuses on various materials that have been developed for use in satellites and rockets. Carbon fibre, in view of its importance, is given primacy of place and the technology of carbon fibre production is dealt with in some detail. The new ceramic materials and their methods of fabrication for high temperature applications (Sailons) and integrated optics (gallium arsenide lithium niobate, etc.) are covered adequately in a easy-to-understand way.

This keynote article is followed by a series c extracts from different technical publications dealin with various developments of materials for use i space as well as materials manufactured in the spac environment. New processing techniques such a rapid solidification, superplastic formation, made-t design composite materials and new alloy develo ments are all covered to varying levels of detail.

The advantages of processing materials in spac such as containerless processing and the absence gravity, are well illustrated through selected extrac that provide information relevant to decisi makers. While the sections on physics of solidific tion, heat transfer and fluid dynamics involved processing materials in space, including the secti on glasses, are quite informative, the tables a charts provide valuable information for top-mana ment decision-makers.

The section on markets gives a good idea of h different countries view space materials process from a commercial viewpoint, even though som the projections appear to be on high side. research guide on major activity centres would be of great use to organizations interested entering this new field. Snippets, such as the sal space beads made by the US National Burea Standards (which is the first commercial sp manufactured product, sold to over 300 orga tions at $386 a piece) make this an emine readable publication.

On the whole the publication provides an excellent overview covering the applications of materials in space systems, materials processing in space and associated research trends/outlook in various institutions the world over. Under the international listing (page 45), the entry for Indian Space Research Organization should read Indian Space Research Organisation (ISRO), Antariksh Bhavan, New BEL Road, Bangalore 560 054.

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The Central Silk Board is implementing the National Sericulture Project at a total cost of Rs. 555.3 crores spread over a period of five years from 1989 with financial assistance from the World Bank and the Swiss Development Co-operation. The collaborators of the project will the Central Silk Board are the five traditional silk-producing States of Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal and Jammu and Kashmir.

The main thrust of the National Sericulture Project is directed towards providing R&D support to the growth of Sericulture and Silk Industry. In order to achieve this objective, the National Sericulture Project provides funds to Universities to support research programmes in Sericulture and Silk Technology. This scheme is centrally administered by the Central Silk Board.

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AN EXPERIMENT AT MOTIVATING YOUNG EARTH SCIENTISTS

In June this year, in a unique experiment, the Department of Science and Technology in cooperation with the Geological Society of India got together a group of young earth scientists (in the age group 30–35 years) who had a proven track record of high quality research and associated them in the process of identifying goals for earth science activity in the coming decade.

The whole object of this exercise of motivating young scientists was based on the principle enunciated by C. V. Raman a long time ago. Based on analyses of the history of science Raman had arrived at the conclusion that great discoveries in science and youthful genius were remarkably closely associated and that 'the principal requisite for means of scientific research was not the maturity of knowledge associated with age and experience, but the freshness of outlook which is the natural attribute of youth'. The conservatism which developed with increasing age was detrimental to great achievements in science. 'The principal function of the older generation of scientific men was to discover talent in the younger generation and to provide ample opportunities for its free expression and expansion.'

The workshop was held at the Geological Society of India in Bangalore on 17 and 18 June. Of seventeen young scientists invited, fifteen responded, and ten actually participated in the meeting. The young scientists were given full freedom to present their viewpoints. Each presentation was followed by a lively discussion and exchange of views between the young scientists and senior participants. The result was that a better perception of the other person's point of view became possible. The discussion provided the seed for many a collaborative programme of research between individual scientists. The main objective of the organisers, which was to bring together persons working in different disciplines and different regions of the country and promote interaction between them, was more than fulfilled.

It was emphasized during the course of the workshop that the Indian continent presented some of the best geological sections in the world, and that it should be possible to identify certain challenging areas and work hard to secure significant results. A review of the proceedings of the workshop was peared in the *Journal of the Geological Society of* *ndia* (vol. 34, no. 2, pp. 215–219).

Taking full note of individual capability, infrastructure facilities available at different centres, and the geological formations that are unique to India, six main topics on which work could be initiated were identified. These are:

Understanding of the plate tectonic processes in the Himalaya and their extension to the Proterozoic and the Archaean

The pre-eminence of the Himalaya as the classic ground for the study of collision tectonics was emphasized. Much team work and sustained effort were necessary to unravel the complicated geological history of the terrain. Palaeomagnetism plays a vital role in the study of these areas. All earth science institutions in the country should get involved in the study. Transects of all collision zones should be attempted. Exchanges of visits and ideas between geologists working in the younger terrain and the considerably older terrains in the south should be encouraged. This will enable a clear perception to be gained of the changing patterns of plate tectonic processes through time.

Understanding of the role of fluids in geological processes

The important role fluids play in metamorphism and mineralization processes has come into prominence in recent years. This field is still new in India and requires to be pursued with vigour. The help of physicists in the IITs and BARC should be availed of in venturing into studies of fluids by adopting the latest techniques of Raman spectroscopy and laser Raman spectroscopy. Groups of individuals and institutions engaged in this study should be identified and given encouragement to pursue the subject more intensively and contribute to our knowledge of metamorphic and mineralization processes.

Study of Proterozoic sedimentary basins

The study of Proterozoic sedimentary basins of India has not received much attention so far. They are a fantastic storehouse of sedimentary structures. Detailed sedimentological studies should receive urgent attention. Geophysical input in defining subsurface characterization, mapping of the basement and seismic profiling to outline the structure are essential. Since, in some sections at least, the sedimentary record as preserved in these basins transgresses the Precambrian–Cambrian boundary, a detailed palaeobiological study should be undertaken. Geologists working in the Himalaya should get deeply involved in the study of these basins in peninsular India.

Study of mafic dyke swarms

India has a priceless heritage of a vast variety of dyke swarms of different kinds and different ages which require to be studied from all angles—petrographical, geochemical, geochronological, palaeomagnetic and structural. They are sure to throw considerable light on the evolution of the Indian lithosphere.

Geochemical characterization of Deccan flood basalts

This is an ongoing research project which has yielded good results. Geochemical characterization and establishment of stratigraphy for the entire Deccan trap region should be attempted. Petrological and isotopic studies should be intensified, as the Deccan flood basalts represent the best candidates for studying crustal contamination in continental flood basalts and continental lithosphere. ^{40}Ar–^{39}Ar age determination should enable precise fixture of the time and duration of the eruption. Correlation of flood basalt eruption with mass extinction is a fruitful field of research which must receive attention. Another fascinating and challenging problem relates to the study of the processes and causes of uplift of the Western Ghats and its possible effect on the drainage pattern of the peninsula.

Studies on groundwater, soil and weather

As a piece of relevant research that has great bearing on the welfare of the people, earth scientists will have to take greater interest in carrying out basic research in respect of groundwater, weathering and soil formation and on the changing patterns of climate.

Groundwater: Processes that cause fluctuation in water table; the manner of recharge and the consequences of large-scale withdrawal; the role played by the vadose zone in transferring water; groundwater contamination, which is posing a serious threat—all these aspects should receive immediate attention.

Soils: A clear understanding of the chemistr
of the weathering process in relation to soi
formation is another important field on whic
research has to be concentrated. Valuable soil i
getting rapidly eroded. Studies should be aime
at controlling this menace, through geomorph
logical appraisal, terracing, contour bundin
afforestation of watersheds and such oth
constructive measures.

Weather: Studies on the changing aspects
climate are equally important. All investigatio
like the study of shallow oceanic sediments in t
shelf region, tree rings, sand dunes, loess a
glaciers should be intensified and knowled
gathered from a study of these aspects periodica
synthesized.

The emphasis of the workshop was on bring
out the best in young earth scientists. An assura
was held out to them on behalf of the Departmen
Science and Technology and the Geological Soci
of India that every effort would be made to supp
their research to contribute significantly to
knowledge of the geology and resources of
country.

B. P. RADHAKRIS

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ABSTRACTS OF TALKS GIVEN AT THE FIFTYFIFTH ANNUAL MEETING OF THE INDIAN ACADEMY OF SCIENCES AT BHOPAL, 10–13 NOVEMBER 1989

Organometallic chemistry and catalysis

S. Bhaduri, *Alchemie Research Centre, P.O. Box No. 155, Thane 400 601*

The relationship between organometallic chemistry and catalysis is presented from a historical viewpoint. Important modern concepts such as catalytic cycles, catalytic intermediates, etc. that define the framework of research in *homogeneous* catalysis are introduced. The justifications for the use of *metal clusters* rather than mononuclear complexes as models for *heterogeneous* catalytic reactions are mentioned.

With this introductory background, results from the author's laboratory on ruthenium and platinum carbonyl cluster-catalysed reactions are presented. For complex cluster-catalysed homogeneous reactions, where detailed kinetic studies are extremely difficult to carry out, the usefulness of model building with well characterized reaction steps is shown. Two reactions, reductive carbonylation of nitrobenzene and transfer hydrogenation of cyclohexenone, are discussed in this context. Finally the use of an anion exchange resin as a support material to generate *heterogenized* cluster-catalysts of novel catalytic activities is discussed.

Instabilities at reacting interfaces in ceramic systems

K. T. Jacob, *Department of Metallurgy, Indian Institute of Science, Bangalore 560 012*

Nonequilibrium heterogeneous systems are frequently encountered in modern ceramics. The interfaces between adjacent phases move during the course of solid state reaction. In binary systems the interface is invariant under isothermal and isobaric conditions. However, in higher order systems the interface is not invariant. Morphological instability of an initially planar interface becomes feasible. Some experimental observations of instability are presented. Although interface energies tend to stabilize moving planar interfaces, this effect is small when the structures of adjacent phases are closely related. In the absence of a simple general principle that can predict morphological instability, the phenomena can be analysed by transport equations of irreversible thermodynamics under appropriate boundary conditions. A parameter can be defined which can distinguish between stable and unstable interfaces. The parameter determines whether or not small perturbations of the interface will grow or decay with time. The parameter is a complex function of all the transport coefficients, starting compositions and chemical potentials of all components in both phases. Because of the complexity of the mathematics involved and the dearth of necessary input data, the search for a more general principle continues.

A set of important boundary conditions at reacting interfaces correspond to the availability of oxide components and oxygen. They determine the degree of flux coupling in ternary and higher-order systems. When oxygen is excluded from one or more interfaces of a ternary compound, oxygen potential gradients develop as a consequence of cationic fluxes. Oxygen pressures of several hundred atmospheres can develop in the interior of reacting systems, giving rise to pore formation and mechanical deterioration of the ceramic.

Intelligent engineering systems

V. V. S. Sarma, *Department of Computer Science and Automation, Indian Institute of Science, Bangalore 560 012*

Intelligent engineering systems represent evolving new generation engineering systems with embedded computer systems whose performance is enhanced by incorporating several levels of human experience, intelligence, expertise and knowledge. Such systems may be characterized by the presence of several of the following attributes:

—They have ability to pursue goals that evolve with time.

—They have ability to capture, store, use and maintain knowledge from diverse sources.

—They are capable of performing intelligence computations, such as those associated with human intelligence involving large numerical and symbolic computations for purposes such as problem solving, reasoning, understanding and learning.

—They are composed of a distributed set of interacting intelligent or conventional subsystems.

—They interact intelligently and intelligibly with human users and other such systems.

—They are adaptive in nature, capable of learning from experience, and capable of allocating their own resources and levels of attention to tasks they perform.

While such systems are clearly futuristic, the present-day revolution in computer technology, artificial intelligence and knowledge-based expert systems holds the promise of supplying solutions to major problems in government and industry in the form of intelligent engineering systems.

Such systems are designed using intelligent computer-aided engineering (ICAE), the goal of which is to construct computer programs that capture a significant portion of an engineer's knowledge, both formal and tacit. Issues that are important are ICAE in design, ICAE in plant operation and maintenance, ICAE in computer-based training, and general ICAE methodology, including progress in areas such as qualitative physics and better understanding of human reasoning.

We shall illustrate the underlying principles of intelligent system design with examples drawn from several projects that are in progress in our Intelligent Systems Laboratory, such as the development of a knowledge-based assistant for natural language requirement analysis, a decision support system for project management, development of a design environment for complex pattern recognition tasks, and development of intelligent subsystems for defence applications.

Some novel one- and two-dimensional methods in high-resolution NMR spectroscopy

S. Subramanian, *Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Madras 600 036*

With the advent of pulsed Fourier transform NMR spectroscopy many new pulse sequences are being developed to unravel complicated high-resolution NMR spectra of large molecules. In homonuclear systems (such as in ^1H NMR) one can use coherence transfer between coupled protons via spin–spin coupling or rotating frame to generate two-dimensional correlation spectroscopy. The present lecture will cover the basic aspects of coherence transfer and principles of 2D spectroscopy and introduce two novel methods of spectral simplification. In one, homonuclear coherence transfer in the rotating frame is used to generate a simplified subspectrum from a more complicated spectrum. In another, homonuclear coherence transfer is used for the 'preparation' and 'mixing' in a two-dimensional pure zero-quantum a pure double quantum correlation spectroscopy useful in studying amide proton–proton connectivities in proteins in 95% aqueous (H_2O) solutions. The above techniques are demonstrated by suitable examples.

Pressure–temperature–time trajectory of granulites from Ganguvarpatti, South India, and its implications on crustal evolution

Anand Mohan, *Department of Geology, Banaras Hindu University, Varanasi 221 005*

The granulites of Ganguvarpatti, which constitute portion of the Archaean South Indian belt, includ silica-deficient sapphirine-bearing granulites, charno ckites, khondalites, gneisses, leptynites and mafi granulites. Numerous interesting reaction textur and coronas including symplectites are well preserve in these granulites. These reaction textures a formed in response to a drop in pressure duri uplift. The frozen-in textural and chemical data a suggestive of low μ H_2O, and coexistence reactants and products in all rocks suggests t chemical potentials of components were buffered mineral assemblages.

These arrested reaction textures, which prese the signature of the different stages of metamorp crystallization, when combined with the pertin models of geothermobarometry indicate a nea isothermal decompressive P–T–t trajectory an fairly rapid uplift of *ca* 15 km. The maxim pressure of 9 kb during the thermal peak indicat 30 km depth of burial during late Archa Considering the average thickness of *ca* 35 km of preserved crust in the southern Indian Shield, crustal thickening of Archaean crust (*ca* 65 km) be explained through the northward subduc during collision and tectonic setting.

Although several other mechanisms are pos for generating the thickened crust, the clockwise P–T–t loops are more likely a consequence of concurrent thermal relaxation and erosion following tectonic crustal thickening in collision belts.

The characteristic swing in the structural trends at the Moyar–Bhavani lineament and the development of the high-grade assemblages suggest that it is a suture zone along which the indentation of Dharwar craton took place. It remains to be seen whether these rocks suggesting hot uplift necessarily means that they formed in single-cycle collisional tectonic setting or there was an Archaean isobaric cooling phase followed by reheating and uplift at a much later time.

X-ray spectroscopic studies of complex oxides

G. Sankar, *Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560 012*

X-ray absorption studies of complex systems containing more than one phase and/or more than one type of coordination have been of recent interest to researchers dealing with catalytic materials, amorphous systems and complex oxides. Procedures to extract structural information and application of X-ray absorption spectroscopy (X-ray absorption near edge structure, XANES, as well as extended X-ray absorption fine structure, EXAFS) to the study of such materials will be illustrated by taking examples of simple oxides like CuO, NiO and related complex systems. Typical complex systems, such as $NiAl_2O_4$, containing more than one type of coordination around the metal ion, as well as the applicability of modified EXAFS function to mixed-phase oxide catalysts Cu/ZnO, Ni/Al_2O_3 and Ni/TiO_2 will be presented.

Siting, energetics and mobility of adsorbates in zeolites

S. Yashonath, *Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560 012*

Zeolites are aluminosilicates with complicated three-dimensional structure consisting of interlinked cages and channels. The inner walls of the cages provide a heterogeneous surface where the molecules are adsorbed and reactions occur, resulting in the well-known catalytic properties of zeolites. The size and shape of the cages, channels and windows are responsible for their shape-selective properties. Extensive experimental investigations, including calorimetric, uptake, IR, Raman, NMR, X-ray, neutron scattering, etc., of organic molecules sorbed in zeolites exist in the literature. However, it is only in recent years that attempts have been made at understanding these interesting systems theoretically.

Molecular dynamics calculations have been carried out on rare gases and small molecules adsorbed in zeolites such as faujasite. Concentration and temperature dependence of the adsorption properties have been investigated. Radial distribution functions, adsorbate–zeolite interaction energy distribution functions, site and cage residence times, diffusion coefficients, cage occupancy, diffusion pathways for cage–cage migration and vibration frequencies have been obtained. It is seen that the adsorbates are rarely found near the centre of the cage. Site-to-site migration of methane in faujasite begins around 150 K. Below this temperature the methanes are localized to the region near the adsorption site. For benzene there are two adsorption sites—the cation and the window site. The motion of the adsorbate in general is highly anisotropic. The calculated vibrational frequencies are in good agreement with neutron scattering data wherever available, and with the grand canonical ensemble Monte Carlo calculation of Rowlinson and coworkers.

Novel aspects of quantum theory of magnetism

G. Baskaran, *Institute of Mathematical Sciences, Tharamani, Madras 600 113*

There has been a renewed interest in the quantum theory of magnetism of insulators following the discovery of high-temperature superconductivity in doped antiferromagnetic insulators. Fascinating connections of some of the problems of magnetism have been made to rather unexpected and parallel developments in fractional quantized Hall effect in condensed matter physics, topological field theory in quantum field theory, and theory of knot polynomials in pure mathematics. I will present some older examples of this phenomenon of parallel developments and argue that historically this is not new. Two specific cases will be discussed at some length. The first one is the phenomenon of pairing and superfluidity occurring in superconductors and ^3He superfluids—this has its parallel in magnetic insulators. Secondly I will discuss how the aforementioned recent parallel developments can be understood in a unified way through the notion of dynamically generated gauge fields in the study of strongly correlated systems introduced by Anderson and the present author.

The physics of fluctuating membranes

Sriram Ramaswamy, *Department of Physics, Indian Institute of Science, Bangalore 560 012*

From the point of view of a physicist, a membrane is a two-dimensional sheet, either open, like a piece of paper, or closed, like the surface of a soap bubble or a red blood cell. While the importance of real biological membranes is well known, physicists are beginning to find that the idealized membranes of which I am going to speak have interesting properties as well.

I shall talk about three classes of systems:

i) Dilute suspensions of closed membranes *without* surface tension, such as red blood cells and quasi-spherical vesicles
ii) The layered phases in oil–water surfactant mixtures
iii) Solutions of two-dimensional polymeric sheets, known as 'tethered surfaces'.

I shall show during my talk that these systems have unusual dynamical properties both near and far from equilibrium. My main results are that all the above systems should have strongly frequency-dependent mechanical properties and that in the layered phases, the application of a sufficiently large velocity gradient should destabilize the phase. I propose various experimental tests of these predictions.

An unconventional mode of repair of DNA damage

R. Jayaraman, *Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021*

Cellular DNA is subject to many insults, some natural and some deliberate. Some of them are dimerization of adjacent pyrimidines, alkylation of bases, depurination/depyrimidination, interstrand cross-linking, oxidative damage, etc. The enteric bacterium *E. coli* has evolved a number of mechanisms to counter the effects of such damages, even at the risk of suffering mutations. The most thoroughly studied process of DNA repair is the SOS response which repairs damages induced by UV, mitomycin C, some alkylating agents, blocks in DNA replication, etc. The SOS response is under the control of two genes, namely *recA* and *lexA*. Mutations in these genes result in hypersensitivity to DNA-damaging treatments. We have observed that in mutants harbouring a combination of two specific mutations, one in the gene encoding the β subunit of RNA polymerase (*rpoB*) and the other in the gene encoding the A subunit of DNA gyrase (*gyrA*), the hypersensitivity to mitomycin C under SOS-negative and excision repair-defective conditions is largely alleviated. The talk will present data that describe this effect and show that the response is distinctly different from the conventional SOS response. An insertion mutation in a hitherto unidentified genetic locus, which we have named *sir*, results in a reduction in the magnitude of the response but does not abolish it. The mechanism of this repair is not understood at present.

Physicochemical mechanisms controlling engineering properties of clays

A. Sridharan, *Department of Civil Engineering, Indian Institute of Science, Bangalore 560 012*

The study deals with the physicochemical mechanisms controlling the engineering behaviour of clays. It is now well established that both attractive and repulsive forces of an electrical nature exist between clay particles and they strongly influence the behaviour of the particles and their aggregations. Repulsive forces between clay particles are primarily attributed to an interaction between double layers. It is of importance to note that this primary force of repulsion increases with an increase in dielectric constant of the medium. The principal contributions to the attractive force come from van der Waals forces and are inversely proportional to the dielectric constant of the medium and the distance between the units.

The conventional 'effective stress' equation should account for the electrical attractive and repulsive forces especially when dealing with cohesive soils. The effective stress equation has been modified to take into consideration the electrical attractive and repulsive forces. Strength and volume change behaviour, the two most important engineering properties, have been shown to be strongly influenced by the electrical forces at particle level with experimental results using fluids of different dielectric constant. The compressiblity/volume change behaviour of clays has been examined in the light of Gouy–Chapman theory of electrical double layer. The effect of hydrated ionic size has been brought out.

It has been shown that the secondary compression coefficient is directly related to the strength of the soil skeleton at particle level which is governed by the modified effective stress. The importance of soil structure/fabric, which is controlled by the electrical forces at particle level, to the physical properties of soils has been brought out.

K-Theory of singular varieties

V. Srinivas, *School of Mathematics, Tata Institute of Fundamental Research, Hobi Bhabha Road, Bombay 400 005*

A *complex vector bundle* of rank n on a space X is a space E, together with a map $p: E \to X$, whose fibres $E_x = p^{-1}(x)$ are complex vector spaces of dimension n, i.e. $E_x \cong \mathrm{C}^n$.

An affine algebraic variety (over C) is the solution set $X \subset \mathrm{C}^n$ of a system of polynomial equations in n complex variables. An *algebraic vector bundle* on X is a complex vector bundle $p: E \to X$ where $E \subset \mathrm{C}^m$ is also an algebraic variety, and p is a polynomial map. K-theory may be used to study vector bundles on an algebraic variety with singularities.

A basic example is given by a *cone*, which is a variety $X \subset \mathrm{C}^3$ defined by $f(x, y, z) = 0$, where f is homogeneous of degree $d \geqslant 2$, and the only singular point of X is the origin $(0, 0, 0)$. It was known that all algebraic vector bundles on X are topologically trivial ($\cong X \times \mathrm{C}^k$ for $k = \mathrm{rank}\ E$), and this was also true algebraically for cones of degrees 2 and 3. However, using K-theory, it was proved that there exist algebraically non-trivial bundles on cones of degree $\geqslant 4$.

Another problem related to cones is the following. Given a $k \times k$ matrix M with determinant 1 whose entries are polynomial functions on a cone X, can it be reduced to the $k \times k$ identity matrix by row and column operations with coefficients which are polynomial functions on X? K-theory may be used to show that for any cone X (even of degrees 2, 3) there exists an M for which this is impossible.

Sex determination as a developmental problem

L. Singh, *Centre for Cellular and Molecular Biology, Hyderabad 500 007*

Primary sex determination offers a model system for unravelling the mysteries of development and evolution. It concerns developmental pathways which, in different species, are variously responsive to environmental, genetic or chromosomal factors. In many species of reptiles (alligators, crocodiles and turtles) ambient temperature during the early stages of embryogenesis determines the sex ratio. In amphibia, however, while it is experimentally possible to interfere with the sex-determining process by, for example, hormone administration or grafting of gonadal rudiments, the sex ratio is normally genetically determined. In other groups, genetic control of primary sex ratio has involved the specialization of the entire sex chromosome in respect of this one function (sex determination), and has led to the virtual loss of all its other genetic functions. The investigation of sex chromosomes therefore offers, arguably, the best strategy for approaching one of the most fundamental question in modern biology: how is development controlled? Sex-determining chromosomes, by definition, are chromosomes of known function. The organ system whose development they control are the gonadal rudiments. Within each species, the sexes afford comparisons both at the developmental and at the DNA level, which facilitate identification of the important differences against the background of features common to both sexes. Moreover, mutations that reverse the sex of individuals offer a uniquely valuable system for the study of developmental control.

Recovery of highly conserved sex chromosome-related, repeated DNA (Bkm), originally detected in a female banded krait, and Y chromosome-specific repeat (M34) from male mouse afford accurate means to study development of gonads. Our novel use of sex chromosome-specific probes has revealed that phenotypic sex of an individual mammal, which is determined by the sex of its gonads (i.e. testes or ovaries), is determined by the presence or absence of a small region of the Y chromosome, located near the centromere of the Y chromosome in the mouse. The testis-determining region of the Y appears to exert its primary effect by directing the supporting-cell lineage of the gonad to differentiate as Sertoli cells, acting cell-autonomously.

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The phenotypic sex of a germ cell, i.e. whether it undergoes spermatogenesis or oogenesis, is determined by whether or not it enters meiotic prophase before birth. This depends not on its own sex chromosome constitution, but on its cellular environment. A germ cell in or near normal testis cords (made up mainly of Sertoli cells) is inhibited from entering meiosis until after birth; one that escapes this inhibition will develop into an oocyte even if it is in a male animal and itself has XY chromosome constitution.

ARTICLES

SYNTHESIS AND EFFECT OF GAMMA IRRADIATION ON SOME NEW 6,8-DICHLORO-4-(3*H*)-QUINAZOLINONES OF BIOLOGICAL INTEREST

Y. A. AMMAR*, Y. A. MOHAMED, N. E. AMIN† and M. M. GHORAB†
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ABSTRACT

Condensation of 2-phenyl-6,8-dichloro-3,1-benzoxazin-4-one (I) with hydrazine hydrate and some sulpha drugs gave IIa and IXa–d respectively. The 3-amino derivative (IIa) was allowed to react with acid anhydrides, phenyl isocyanate or isothiocyanate and aromatic aldehydes as well as arylsulphonyl chloride to give IVa, b, Va, b, VIa–d and VIIIa, b respectively. Thiazolidinones (VIIa, b) were obtained by the reaction of VIa, c with mercaptoacetic acid. The effect of gamma irradiation on and antibacterial activity of some of the synthesized compounds are described here.

INTRODUCTION

Various 4(3*H*)-quinazolinones have been investigated for biological and pharmacological activities[1-4]. The halogenated derivatives are reported to possess tranquilizer and hypnotic activity[5]. In view of these findings, we undertook the synthesis and evaluation of antimicrobial activity of some novel 6,8-dichloro-2-phenyl-4(3*H*)-quinazolinones. The effect of gamma irradiation on some of these compounds was also studied.

RESULTS AND DISCUSSION

Reaction of 6,8-dichloro-2-phenyl-3,1-benzoxazin-4-one (I)[6] with hydrazine hydrate gave 3-amino-6,8-dichloro-2-phenyl-4(3*H*)-quinazolinone (IIa) as a main product, with the formation of III. Compound III was confirmed through its synthesis by fusion of I with the 3-amino derivative.

The structure of II was confirmed on the basis of analytical data and IR spectrum which showed NH$_2$ bands as double peaks at 3300 and 3320 cm^{-1}. IR spectrum of III showed disappearance of NH$_2$ bands. Similarly I underwent condensation with benzoylhydrazine to give IIb. Its IR spectrum showed bands at 3250 (NH), and 1720 and 1680 cm^{-1} (CO). On the other hand IIb can also be synthesized by the reaction of IIa with benzoyl chloride in dry pyridine.

Compound IIa was found to react with phthalic anhydride or tetrabromophthalic anhydride to give the phthalimido derivatives (IVa, b). The structure of IV was established from elemental analyses and IR measurements which revealed the presence of CO (1760, 1740 and 1700 cm^{-1}).

The hypoglycaemic activity[7] of urea and thiourea derivatives led us to react IIa with phenyl isocyanate or isothiocyanate to obtain the corresponding urea and thiourea derivatives (Va, b). Structure elucidation of V was based on IR spectra, which exhibited bands at 3400, 3350 (NH), 1720, 1680 cm^{-1} (C=O) for Va, while for Vb the C=S band appeared at 1340 cm^{-1}. The UV spectrum of Vb in DMF showed two sets of bands at λ_{max} 270, 315 nm (log ε at 4.11, 4.19).

The hydrazone derivatives (VIa–d) were formed through condensation of IIa with aromatic aldehydes. IR spectra of VI revealed the disappearance of NH$_2$ bands characteristic for the parent compound (IIa). Cyclization of VIa, c with mercaptoacetic acid gave the thiazolidinones (VIIa, b). IR spectra exhibited bands at 1710 and 1680 cm^{-1} (C=O).

Sulphonamides are drugs of proven therapeutic importance[9]. Therefore it was considered of interest to incorporate the sulphonamido moiety in the quinazolinone nucleus by two methods. Condensation of the 3-amino derivative (IIa) with arylsulphonyl chloride gave the sulphonamido derivatives (VIIIa, b). IR spectra displayed characteristic absorption bands

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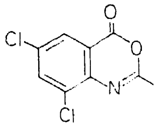
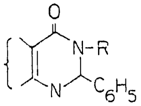
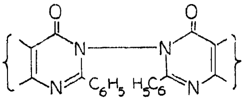
*For correspondence.



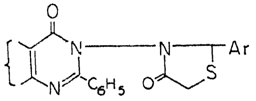
I



IIa: $R=NH_2$
IIb: $R=NHCOC_6H_5$
IVa: R = Phthalimido
IVb: R = Tetrabromophthalimido
Va: $R=NHCONHC_6H_5$
Vb: $R=NHCSNHC_6H_5$
VIa: $R=C_6H_5$
VIb: $R=C_6H_4\cdot CH_3\text{-}p$
VIc: $R=C_6H_4\cdot OCH_3\text{-}p$
VId: $R=C_6H_4\cdot NO_2\text{-}p$
VIIIa: $R=NHO_2SC_6H_4\cdot Cl\text{-}p$
VIIIb: $R=NHO_2SC_6H_4\cdot CH_3\text{-}p$
IXa: R = Sulphaguanidinyl
IXb: R = Sulphapyridinyl
IXc: R = Sulphadimidinyl
IXd: R = Sulphathiazolyl



III



VII

VIIa: $Ar=C_6H_5$
VIIb: $Ar=C_6H_4\cdot OCH_3\text{-}p$.

at 3200 ($NHSO_2$) and 1710 cm^{-1} (C = O). The PMR spectrum of VIIIb in DMSO-d_6 exhibited signals at 2.3 (3H, *s*, CH_3), 4.3 (1H, hump NH; D_2O exchangeable) and 7.1–8.2 ppm (11H, *m*, Ar–H). Another type of sulphonamido derivatives (IXa–d) were obtained through condensation of I with sulpha drugs. IR spectra showed bands at 3150 ($NHSO_2$) and 1700 cm^{-1} (C = O).

Effect of gamma irradiation on some of the synthesized compounds

Nine compounds (IIa, III, IVa, IVb, Va, Vb, VIIIa, IXb and IXd) were irradiated in dimethylformamide (III, IXb and IXd at 5×10^{-5} M; the rest at 1×10^{-4} M) using the well-known chemical method with ferrous sulphate as dosimeter[9]. Irradiation was carried out at room temperature in the presence of atmospheric oxygen. Several doses between 5 and 200 Gy were used.

Ultraviolet spectra of all the irradiated compounds except IVb did not reveal major changes. Compound IVb gave a greatly altered UV absorption profile after irradiation with 200 Gy.

Antibacterial activity

Most of the synthesized compounds were screened for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus*. The procedure was according to Ammar *et al.*[10] Minimum inhibitory concentration (MIC) was determined by the dilution assay technique of Lechevalier *et al.*[11] Assay plates were incubated at 37°C for one day.

Only two compounds (VIIIa, b) showed activity against all three tested organisms while the other tested compounds showed activity against one or two of the tested organisms. This indicates that incorporation of the sulphonamido moiety with the quinazoline nucleus confers higher activity against the tested organisms. The results are given in table 1.

EXPERIMENTAL

Melting points were determined on electrothermal melting point apparatus and are uncorrected. Microanalyses were carried out at the Microanalytical Data Unit, Cairo University, on a Heraeus instrument. IR spectra were recorded on a Pye-Unicam Sp-1200 and Sp-1000 using KBr technique. UV and visible absorption spectra were recorded using a Beckman

Table 1 *Antimicrobial activity of some 6,8-dichloro-2-phenyl-3-substituted-4(3H)-quinazolinones*

	Minimum inhibitory concentration (μg/ml)		
Compound	*E. coli*	*Staph. aureus*	*Streptococcus*
IIa	+(100)	–	–
IIb	–	–	–
III	–	–	+(100)
IVa	–	–	+(100)
IVb	–	–	+(100)
Va	–	+(100)	–
Vb	–	–	+(100)
VIa	–	–	+(100)
VIb	+(100)	–	–
VIc	+(100)	+(100)	–
VId	+ +(50)	–	–
VIIIa	+ + +(30)	+ +(50)	+(100)
VIIIb	+(100)	+(100)	+(100)
IXa	+ +(50)	–	–
IXb	–	–	+(100)
IXc	–	+(100)	+ +(50)

The number of '+' is proportional with activity; '–' indicates no activity.

double beam spectrophotometer model 24 in the range 190 to 700 nm. PMR spectra were recorded on a Varian EM–360 L 60 MHz spectrometer using TMS as internal standard. Gamma irradiation source was a Gamma Cell 40, which is caesium-137.

Condensation of I with hydrazines was carried out according to the reported method[11]. Physicochemical data for the synthesized compounds are given in table 2.

3-Benzoylamino-2-phenyl-6, 8-dichloro-4(3H)-quinazolinone (IIb)

To a solution of IIa (0.01 mol) in pyridine (30 ml), benzoyl chloride (0.01 mol) was added and the reaction mixture was refluxed for 15 min to give IIb.

Preparation III

A mixture of I (0.01 mol), the 3-amino derivative (IIa; 0.01 mol) and anhydrous $ZnCl_2$ (0.5 g) was fused at 160°C for half an hour to give III.

Reaction of IIa with phthalic anhydride or tetrabromophthalic anhydride

A mixture of IIa (0.01 mol) and phthalic anhydride or tetrabromophthalic anhydride (0.01 mol) in acetic acid (50 ml) was refluxed for 5 h to give IVa, b.

Table 2 *Physicochemical data for 6,8-dichloro-2-phenyl-3-substituted-4-(3H)-quinazolinones*

Compound	M.P. (°C)	Cryst. solvent	Yield (%)	Mol. formula	Analysis (%) Required/Found		
					C	H	N
IIa	200	E	79	$C_{14}H_9N_3OCl_2$	54.90 54.95	2.94 2.90	13.72 13.80
IIb	219	E	80	$C_{21}H_{13}N_3O_2Cl_2$	61.46 61.50	3.17 3.25	10.24 10.35
III	180	E/W	35	$C_{28}H_{14}N_4O_2Cl_4$	57.93 57.98	2.41 2.56	9.65 9.75
IVa	232	A	72	$C_{22}H_{11}N_3O_3Cl_2$	60.55 60.67	2.52 2.71	9.63 9.70
IVb	250	A	75	$C_{22}H_7N_3O_3Br_4Cl_2$	35.10 35.15	0.93 1.00	5.59 5.62
Va	235	E	59	$C_{21}H_{14}N_4O_2Cl_2$	59.29 59.32	3.29 3.35	13.12 13.25
Vb	228	E	54	$C_{21}H_{14}N_4OSCl_2$	57.14 57.10	3.17 3.15	12.69 12.75
VIa	158	E	81	$C_{21}H_{13}N_3OCl_2$	63.95 63.87	3.29 3.25	10.65 10.60
VIb	218	E	86	$C_{22}H_{15}N_3OCl_2$	64.70 64.75	3.67 3.62	10.29 10.35
VIc	228	E	80	$C_{22}H_{15}N_3O_2Cl_2$	62.26 62.30	3.53 3.60	9.90 9.85
VId	234	E	79	$C_{21}H_{12}N_4O_3Cl_2$	57.40 57.45	2.73 2.80	12.75 12.80
VIIa	230	E	42	$C_{23}H_{15}N_3O_2SCl_2$	58.97 58.90	3.21 3.25	8.97 8.85
VIIb	250	E	46	$C_{24}H_{17}N_3O_3SCl_2$	57.83 57.92	3.41 3.54	8.34 8.42
VIIIa	142	E	63	$C_{20}H_{12}N_3O_3SCl_3$	49.94 49.87	2.49 2.52	8.74 8.81
VIIIb	194	E	66	$C_{21}H_{15}N_3O_3SCl_2$	54.78 54.68	3.26 3.30	9.13 9.12
IXa	136	A	72	$C_{25}H_{16}N_4O_3SCl_2$	57.36 57.40	3.06 3.10	10.70 10.63
IXb	238	A	78	$C_{21}H_{15}N_5O_3SCl_2$	51.64 51.70	3.07 3.12	14.34 14.42
IXc	202	A	69	$C_{26}H_{19}N_5O_3SCl_2$	56.52 56.64	3.44 3.52	12.68 12.60
IXd	260	A	71	$C_{23}H_{14}N_4O_3S_2Cl_2$	52.17 52.25	2.64 2.70	10.58 10.65

E, Ethanol; W, water; A, acetic acid.

Reaction of IIa with phenyl isocyanate or isothiocyanate

To a solution of IIa (0.01 mol) in benzene (50 ml) phenyl isocyanate or isothiocyanate (0.01 mol) was added. The reaction mixture was refluxed for 3 h to give VIa, b.

Condensation of IIa with aromatic aldehydes

This was done according to the reported method[11].

Cyclization of IVa, c with mercaptoacetic acid

A solution of IVa, c (0.01 mol) in benzene (50 ml) was treated with mercaptoacetic acid (0.01 mol). The mixture was refluxed for 12 h to give VIIa, b.

Condensation of IIa with arylsulphonyl chlorides

A mixture of IIa (0.01 mol), the requisite arylsulphonyl chloride (0.01 mol) and triethylamine (0.5 ml) in benzene (50 ml) was refluxed for 3 h to give VIIIa, b.

3-(4-N-substituted sulphonamido)-phenyl-6, 8-dichloro-2-phenyl-4(3H)-quinazolinones (IXa–d)

These were prepared according to the reported method[12].

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SEPARATION BY PULSED-FIELD GRADIENT GEL ELECTROPHORESIS OF GIANT LINEAR PLASMIDS FROM ANTIBIOTIC-PRODUCING STRAINS OF *STREPTOMYCES* AND *NOCARDIA*

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ABSTRACT

Eleven strains belonging to either *Streptomyces* or *Nocardia* were examined for the presence of giant linear plasmid(s) by pulsed-field gel electrophoresis with a 'home-made' apparatus. Such a plasmid could be detected only in *S. coelicolor* (where its presence is already known) and in *S. rimosus*. The size of the *S. rimosus* plasmid was estimated to be 255 kb, which makes it somewhat smaller than the *S. coelicolor* 320 kb plasmid. Nick-translated *S. rimosus* plasmid DNA hybridizes mainly with the plasmid and faintly with the chromosomal DNA. It does not hybridize with either the chromosomal or plasmid DNA of *S. coelicolor*, suggesting that the latter is an altogether different plasmid. Since *S. rimosus* is an antibiotic-producing strain, there is a possibility, if not the likelihood, that the genes for antibiotic production may be located on the giant plasmid detected.

INTRODUCTION

In recent years there have been significant developments in the technique of gel electrophoresis that allow separation of large DNA fragments[1-4]. These new methods use two electric fields applied alternately at an angle greater than 90°. Among these, pulsed-field gradient gel electrophoresis (PFGGE) has been proved to be very effective in separating very large DNA molecules[5,6]. The added advantage in this method comes from the fact that the electric fields used are inhomogeneous, so that the leading edge of the DNA band faces a weaker field than the trailing edge. This results in band compression and relatively narrow DNA bands are obtained. However, this technique also suffers from a drawback; the DNA samples in different lanes tend to fan out and direct comparison of DNA size may be difficult in some cases. This problem can be overcome to a large extent by making the negative electrodes much larger than the gel size. We have built a PFGGE apparatus in our laboratory which uses 30 cm cathodes for a 10 × 10 cm gel (see next section).

These developments in electrophoresis technique and newer and milder methods of DNA sample preparation[7] have allowed the study of the complete DNA contents of cells. Kinashi and coworkers[8] have used orthogonal field alternation gel electrophoresis (OFAGE) to separate giant linear plasmids in some of the antibiotic-producing strains of *Streptomyces*. In spite of genetic evidence[9-11] for the involvement of linear plasmid SCP1 of *S. coelicolor* in the production of methylenomycin, it could not be separated by conventional methods such as gel electrophoresis and CsCl–EtBr ultracentrifugation, or visualized by electron microscopy. Using OFAGE Kinashi *et al.* could separate DNA bands corresponding to giant linear plasmids from *S. coelicolor* and also from two antibiotic-producing strains of *S. lasaliensis*. Furthermore, no large plasmid was seen in the non-producer strain PR2-45 of *S. lasaliensis*. In another study Kinashi and Shimaji[12] have reported that only six out of 12 antibiotic-producing strains of *Streptomyces* tested showed giant linear plasmids. Characterization and understanding the role of these plasmids is of interest in both basic and applied research. As a first step in this direction we have tested 11 antibiotic producers for the presence of giant plasmids using a PFGGE apparatus built in our laboratory. Surprisingly, only two out of the 11 strains studied showed the presence of giant plasmids. We have used lambda *vir* DNA ladder as standards to estimate the size of these plasmids. We also describe, in this communication, the design of the PFGGE apparatus used, which can be made from relatively inexpensive components.

MATERIALS AND METHODS

Pulsed-field gradient gel electrophoresis

The PFGGE apparatus used in this study was

made according to the basic design of Smith *et al.*[13], with some modifications. The top view of the apparatus is shown in figure 1. T is the buffer tank made from acrylic sheets (35 × 35 cm). The bottom of the tank is jacketed so that chilled water (from a refrigerated circulating water bath) may be circulated to cool the buffer during electrophoresis. The electrodes A^+, A^- and B^+, B^- generate the two inhomogeneous electric fields. While the anodes (A^+ and B^+) are single platinum wires (1 mm dia.) mounted vertically, the cathodes (A^- and B^-) consist of arrays of vertical platinum wires (0.2 mm dia) mounted at 1 cm separation. All the wires in one cathode are connected through diodes (1 N 4007) to a common cathode (only end diodes are shown in the figure). Power to the electrodes is supplied from a 0–500 VDC power pack through a pair of timer-controlled high-voltage DC relays as shown in the figure. The gel G (10 × 10 cm) is
in the top left-hand corner, with the wells orien
shown by the dotted line in the figure.

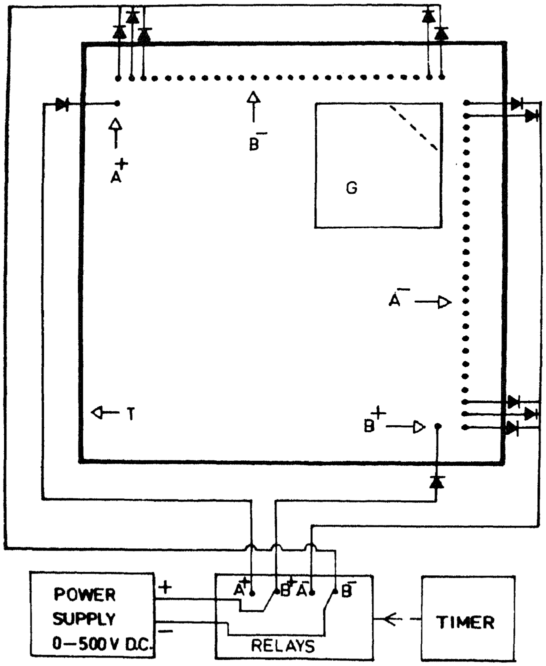

Figure 1. Top view of PFGGE apparatus. The electrodes (A^+, A^- and B^+, B^-) are mounted vertically in the buffer tank T (35 × 35 cm). The gel G (10 × 10 cm), with the wells in the upper right corner (dotted line), is placed in one corner of the tank as shown. The electrodes are isolated by diodes (1 N 4007) as shown. Only end diodes are shown for the cathodes in the figure. Connection to the power pack (0–500 V DC) is made through a pair of timer-controlled high-voltage DC relays as shown.

Electrophoresis was carried out in 0.5
buffer (89 mM Tris, 89 mM boric acid,
EDTA). The temperature of the buffer was
tained at 18°C by circulating chilled water/a
mixture through the jacket in the bottom
buffer tank. After electrophoresis the gel was s
with ethidium bromide (1 μg/ml), washed
distilled water, and viewed with a short-wave
UV transilluminator.

Preparation of DNA samples

Cultures were grown for 2 days at 30°C with
aeration by inoculating 10^5 spores in 50 ml
extract–malt extract medium containing, in 1
0.3 g yeast extract (Difco), 1 g glucose, 34 g su
0.2 ml of 2.5 M $MgCl_2$ and 2.5 ml of 20% g
The mycelium was harvested by centrifugatio
washed twice with P buffer (10.3 g sucrose,

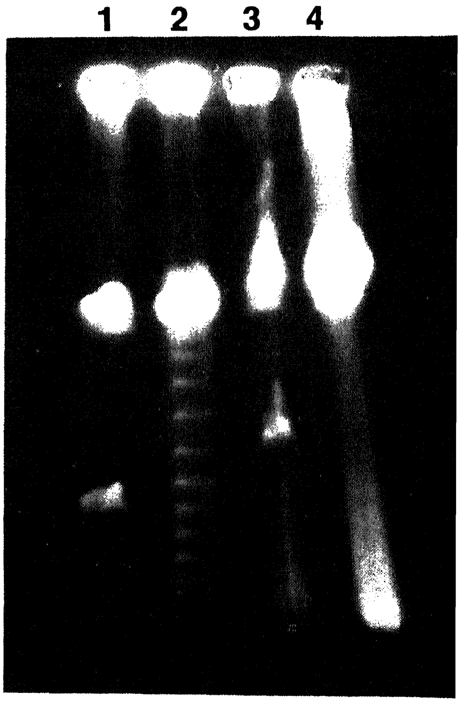

Figure 2. PFGGE analysis of *S. rimosus* (lane
coelicolor M130 (lane 4), *S. coelicolor* M138 (la
DNA. Lane 2 has lambda *vir* DNA ladd
standards.

K_2SO_4, 200 mg $MgCl_2 . 6H_2O$, 0.2 ml trace element solution[14], 1 ml of 0.5% KH_2PO_4, 10 ml of 3.68% $CaCl_2 . 2H_2O$ and 1.6 ml of 0.1 M Tris–HCl, pH 8.0, in 100 ml). After washing the mycelium was suspended in 2 ml of P buffer and added to a solution containing 2 ml of 0.5 M EDTA and 100 μl lysozyme solution (30 mg/ml) in a small petri plate (2 inch dia.). To this, 2 ml of 1.5% low-melting-point agarose (Sigma) kept at 37°C was added and mixed. After solidification of the agarose, it was covered with 1 ml of 0.5 M EDTA (in P buffer) and the plate was incubated overnight at 37°C. The agarose was then cut into small slices (5 × 5 mm) and incubated in 100 ml of 5% sodium dodecyl sulphate solution at 50°C for 1 day with gentle shaking. The slices were then treated with 5 ml of RNase solution (100 μg/ml, containing 100 mM EDTA, heat-treated for 5 min at 80°C) for 1 day at 37°C and then with pronase (Sigma) solution (2.5 mg/ml) containing 100 mM EDTA for 1 day at 37°C. The slices were then washed and stored in 100 mM EDTA at 4°C till use.

^{32}P labelling of DNA and Southern hybridization

DNA probes were labelled with ^{32}P using a nick translation kit (Amersham). Transfer of DNA from gel to nitrocellulose filter and Southern hybridization were carried out according to the method of Maniatis *et al.*[15] Autoradiography was done by exposing the filter to X-ray film with intensifying screens for five days at −70°C.

RESULTS AND DISCUSSION

In all eleven antibiotic-producing strains were investigated. These were *S. rimosus* (NCL), *S. noursei* 2424, *S. fradiae* 2419, *S. aureofaciens* 2616 (from National Chemical Laboratory, Pune, India); *S.*

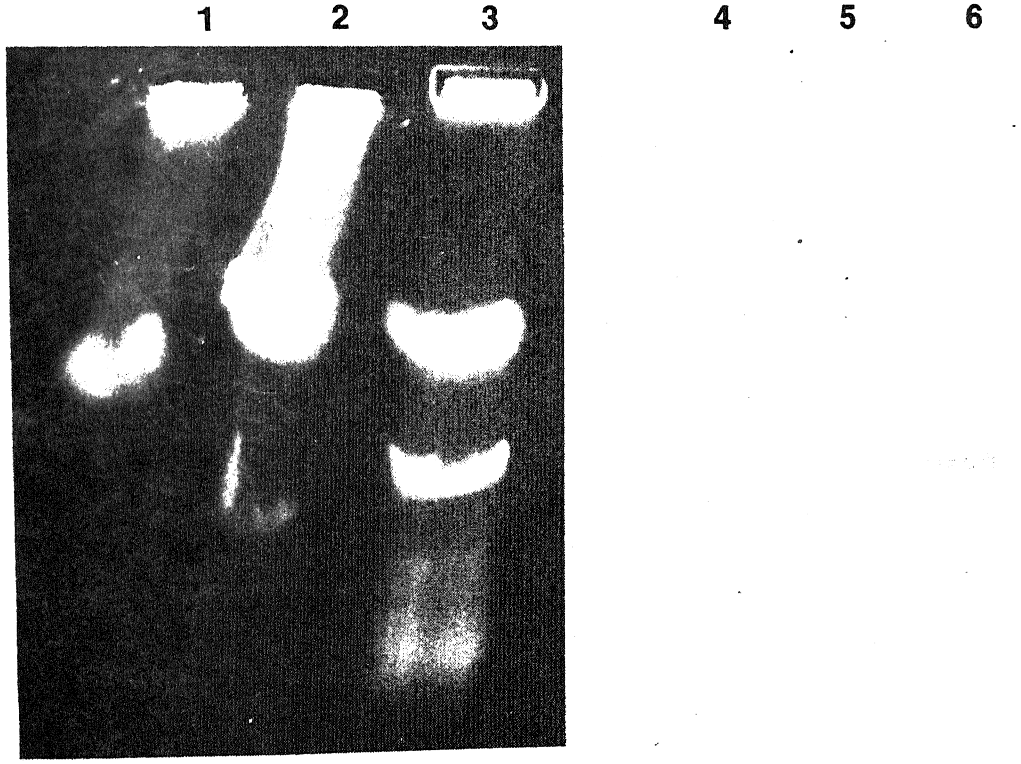

Figure 3. PFGGE (lanes 1 to 3) and Southern blot (lanes 4 to 6) analysis for *S. coelicolor* M138 plasmid. Lanes 1 and 4, *S. coelicolor* M130; lanes 2 and 5, *S. rimosus*; lanes 3 and 6, *S. coelicolor* M138. The probe was ^{32}P-labelled pBR322::mmr containing methylenomycin resistance gene from *S. coelicolor* A3(2).

azureus 2726, *S. coelicolor* M138, *S. coelicolor* M130 (from John Innes Institute, England); *S. griseus* A39, *S. olivaceus* ATCC 11626, *Nocardia* sp. Al-R 1493 and *Nocardia* sp. Of these only *S. coelicolor* M138 and *S. rimosus* showed the presence of giant plasmids. *S. coelicolor* M138 is known to have the linear plasmid SCP1 (refs. 9–11). This plasmid has also been separated by Kinashi *et al.*[8] by OFAGE. We have used this strain to standardize the operation of our PFGGE apparatus. Figure 2 shows the PFGGE analysis of *S. rimosus* (lane 1), *S. coelicolor* M130 (lane 4) and *S. coelicolor* M138 (lane 3) DNA. The lambda DNA ladder (generous gift from Prof. C. R. Cantor, USA) in lane 2 provides standards of linear DNA fragments; successive bands are separated by 42.5 kb. The strain *S. coelicolor* M130 is known to lack giant linear plasmids and, therefore, was used as a control. The electrophoresis was carried out for 70 h at an electric field strength of 5 V/cm and pulse time of 120 s. under these conditions good separation could be obtained for DNA fragment size up to about 500 kb, as can be seen from the ladder (the range of DNA size separation in PFGGE is very strongly dependent on the electric field strength and pulse time). DNA molecules of higher molecular size comigrate under the conditions used, and appear as bright spots near the wells. In lanes 1, 3 and 4 these spots are due to chromosomes and in lane 2 the spot represents higher lambda DNA polymers which could not be separated. Lanes 1 and 3 each show a bright band of DNA near the front edge of the gel. These bands correspond to giant plasmids in *S. rimosus* and *S. coelicolor* M138 respectively. The plasmid in *S. rimosus* is slightly smaller than that in *S. coelicolor*. By comparing the positions of plasmid bands with bands of the lambda ladder, the size of *S. rimosus* plasmid was estimated to be 255 kb and that of *S. coelicolor* M138 as 320 kb. To check linearity of the plasmids, the samples were run under different

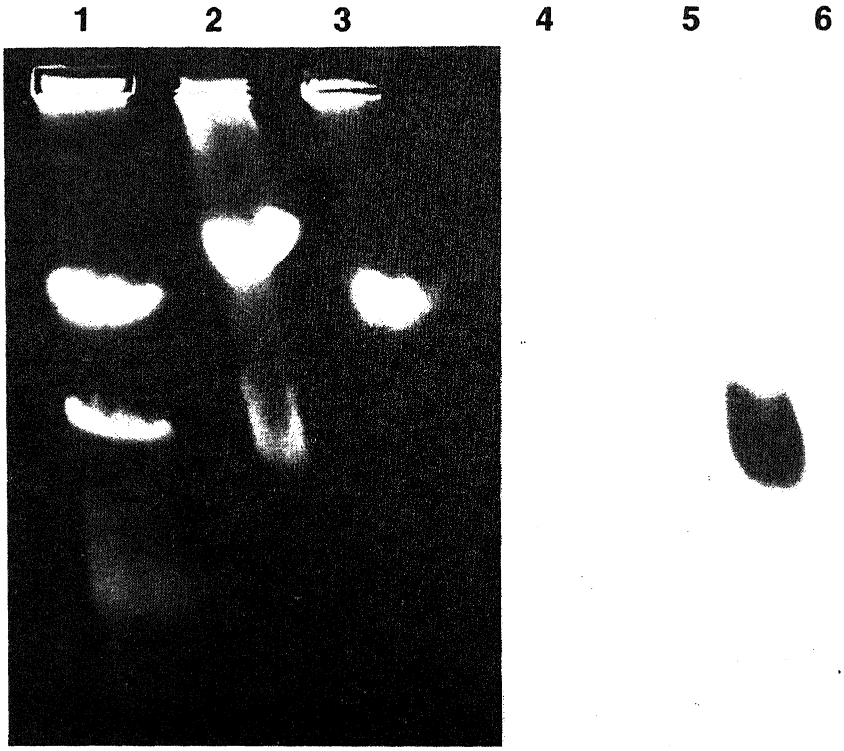

Figure 4. PFGGE (lanes 1 to 3) and Southern blot (lanes 4 to 6) analysis for *S. rimosus* plasmid. Lanes 1 and 4, *S. coelicolor* M138; lanes 2 and 5, *S. rimosus*; lanes 3 and 6, *S. coelicolor* M130. The probe was ^{32}P-labelled *S. rimosus* plasmid.

electrophoresis conditions, such as field strength 10 V/cm and pulse time 30 s. The relative position of plasmids with respect to bands of the lambda ladder remained unaffected. This is possible only if the plasmid DNA is linear, like the DNA in the lambda ladder, since it is well known that movement of covalently closed circular DNA and that of linear DNA are drastically different in PFGGE[16].

Figure 3 shows Southern analysis of DNA from *S. coelicolor* M138, *S. coelicolor* M130 and *S. rimosus*. The probe pBR 322::mmr containing the methylenomycin resistance gene from *S. coelicolor* A3(2) (gift from Prof. Keith Chater, John Innes Institute, England), labelled with ^{32}P by nick translation, hybridized with the plasmid band of *S. coelicolor* M138. This is consistent with the result of Kinashi *et al*. Figure 3 also shows that the probe does not have any homology with either the chromosome of *S. coelicolor* M138 or with any other DNA band seen in the figure.

In the case of *S. rimosus* we have investigated the homology between the plasmid and the chromosome. The plasmid from *S. rimosus* was electroeluted from a PFGGE gel. It was then labelled with ^{32}P by nick translation and used as a probe for Southern hybridization. Figure 4 shows Southern analysis using the labelled *S. rimosus* plasmid. The probe hybridized with the corresponding plasmid band as expected. However, it also showed weak, but distinct, binding with the chromosome band and at the well. The weak binding at the well may be due to some plasmid trapped in the cell debris in the sample. Binding of the probe with the chromosome, however, means that either the plasmid also exists integrated within the chromosome or it shares some homology with the chromosome. More work is required to check these possibilities.

From our analysis it is clear that the production of antibiotics is not always associated with the presence of giant plasmids. In fact, so far, only a small number of antibiotic producers have shown the presence of giant plasmids. Even in the strains in which plasmids have been detected, their role in antibiotic production has not been directly demonstrated (except in *S. coelicolor*). We are attempting to cure the *S. rimosus* plasmid to investigate if it is involved in the production of tetracycline. These experiments are in progress.

ACKNOWLEDGEMENTS

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PETROCHEMISTRY OF THE GRANITE NEAR VANDANMEDU, IDUKKI DISTRICT, KERALA, INDIA

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ABSTRACT

The present study deals with the discordant granite pluton near Vandanmedu in Idukki district of Kerala, emplaced within the Precambrian metamorphics. Mineralogical and textural relations categorize the rock as granite. K_2O/Na_2O ratio indicates granite–adamellite range and A/CNK value its peraluminous nature. K/Rb ratio suggests Rb depletion tendency and the major, trace element variations and critical trace element ratios Rb/Sr and Ba/Sr, suggest limited crystal fractionation. SiO_2, Na_2O+K_2O levels and Fe/Mg, Ga/Al ratios point to an A-type affinity. Relation of Ga/Al vs major oxides and agpaitic index further confirm the A-type nature. Since A-type granites are likely sources for Sn, Mo, Nb, W, Ta and F, further studies on these lines are suggested.

INTRODUCTION

THE Kerala region, forming part of the western continental margin of India, presents evidence of a tectogenetic episode during the Late-Precambrian–Early Palaeozoic period with associated rifting and granitic activity[1-4]. Several bodies of intrusive granites associated with this activity have been reported from this region. The granite of the present study occurs as a circular pluton covering an area of about 8 km^2 near Vandanmedu, in Idukki district of Kerala (figure 1).

GEOLOGICAL SETTING

The granite is medium to coarse-grained, emplaced within Precambrian crystallines which comprise biotite–hornblende gneisses (migmatite) and charnockite (figure 1). The granite body shows sharp contact with the surrounding country rocks. The regional strike is NNE with dips of 45–60° toward ESE. However, at places, due to development of marginal foliation and presence of pink appurtenances of granite in surrounding migmatite, the contact appears gradational.

There does not seem to be any disturbance of the structures in the host rock due to the emplacement of granite. This passive role of the country rock during emplacement coupled with the sinuous contacts, lack of internal structure, irregular shape and minor stoping structures at the border, categorises the pluton as a discordant type[5]. Small enclaves of biotite gneiss with locally diffused contacts are observed within the granite especially towards the periphery. The surrounding country rocks are polymetamorphosed with the biotite–hornblende gneiss indicating a retrogressive amphibolite facies overprinted on the original granulite facies prevalent in the area[6,7]. The age of the granite is not known. However, considering the occurrence of similar granites in other parts of Kerala region[2,4,8,9], a Late-Precambrian–Early Palaeozoic correlation is suggested for this granite.

PETROGRAPHY

The chief mineral constituents are K-feldspar, plagioclase feldspar, quartz, biotite ± hornblende; set in a hypidiomorphic granular texture. The predominating alkali-feldspar is the perthite variety of microcline. The plagioclase feldspar is myrmekitized and often corroded by the alkali-feldspar. Determination using universal stage on 24 grains of plagioclase indicates a range in composition from An_{95}–Ab_5 to An_{85}–Ab_{15}. The quartz occurs as anhedral grains with tongues projecting into adjacent mineral phases partially engulfing them. In addition, quartz occurs as small droplets within potash feldspar, small interstitial grains with irregular shape and also as myrmekitic quartz. Laths of brown pleochroic biotite constitute the major mafic component. Hornblende is very limited and often altered to granular aggregate with associated iron ores. Sphene and apatite are the chief accessories.

The modal composition of 8 samples, when plotted in the Q–A–P triangular diagram[10] falls in the granite field (figure 2). The isotropic nature of

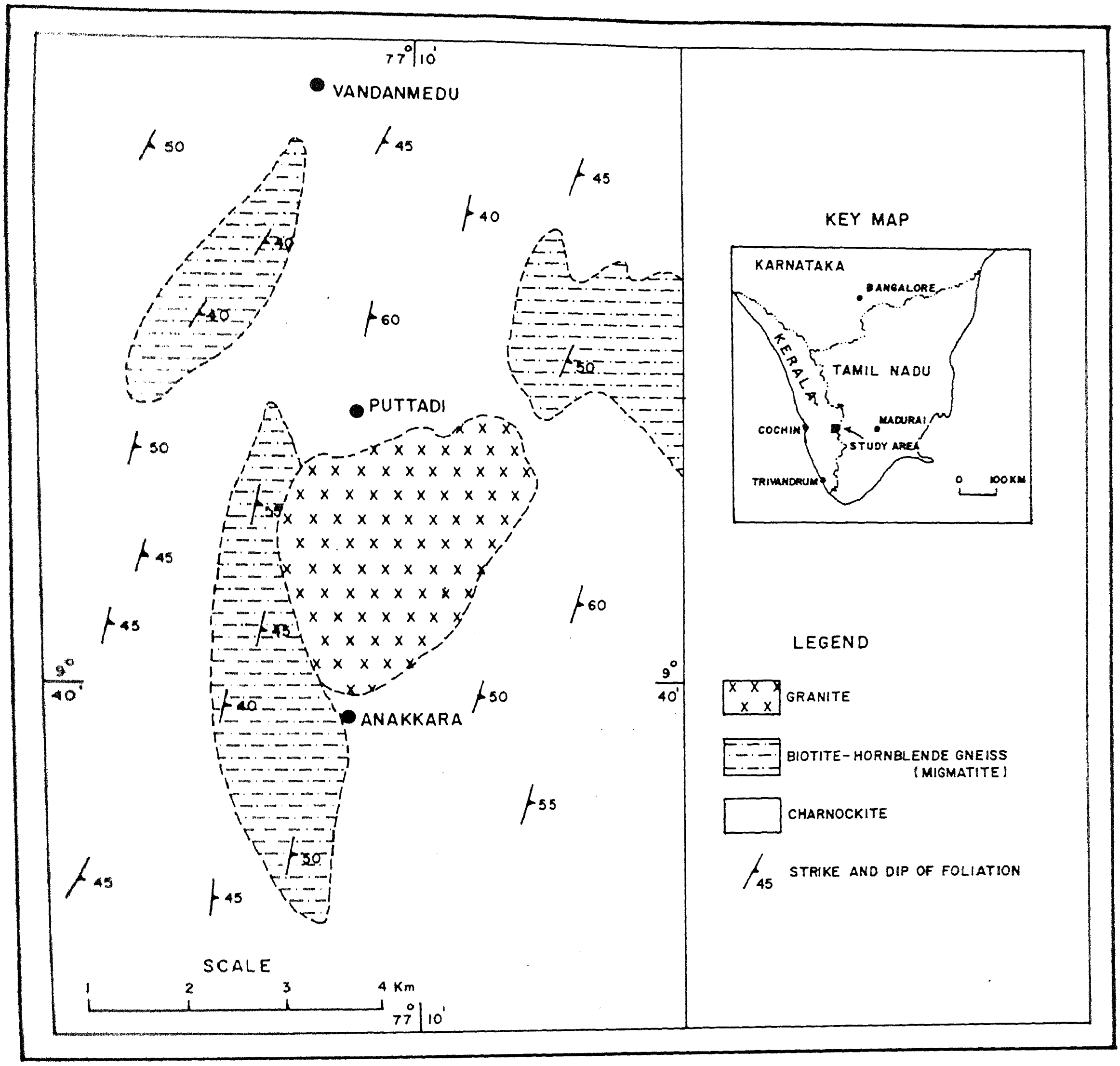

Figure 1. Geological map.

fabric and absence of strain induced recrystallization are suggestive of post-tectonic emplacement. The lower An content of plagioclase feldspar is a feature commonly noted in post-kinematic granites[11].

GEOCHEMISTRY

The chemical analysis of eight samples from the granite are presented in table 1. Pulverized (−200 mesh) and homogenized samples were analysed for major elements by the conventional wet chemical method, trace elements, Na and K by AAS. Georeference standards Sy_2, Sy_3 and MRG_1 were used to check precision. The analytical uncertainties are, for SiO_2 about 1%, Al_2O_3 and FeO about 2% and for the rest of major elements about 5% of the amount present. Regarding trace elements, the analytical uncertainty for concentration more than 100 ppm is better than 4% and for lesser concentrations about 10% of the amount present.

The SiO_2 and Al_2O_3 values indicate a granite to granodioritic nature. The K_2O/Na_2O values are

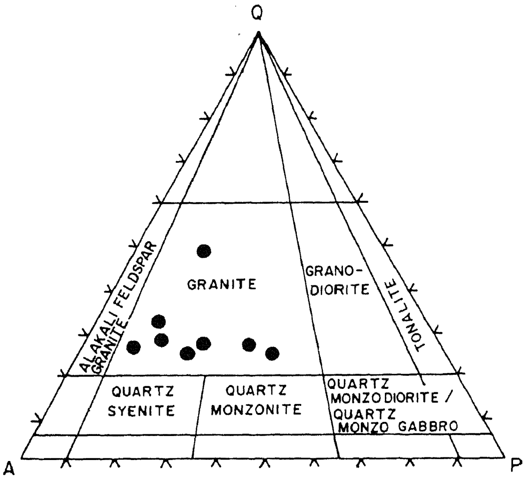

Figure 2. Q–A–P Triangular diagram[10].

generally above unity bringing out the potassic nature of the granite. The higher level of alkalies with dominance of K_2O (av. 4.49%) is a typical geochemical character noted in the post-tectonic granites of this region[12]. Based on K_2O/Na_2O ratios[13] the rock type chemically show granite–adamellite range (figure 3). Since the A/CNK values

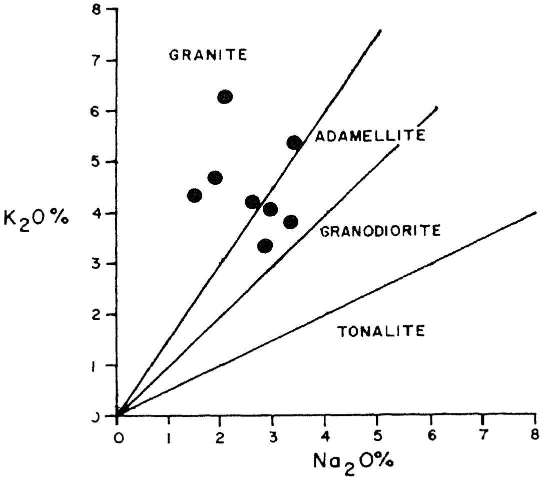

Figure 3. K_2O vs Na_2O diagram[13].

Table 1 *Chemical composition of eight samples of granites from Vandanmedu*

Sample No.	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	Gr 6	Gr 7	Gr 8	Range	Mean	SD
SiO_2	71.05	76.54	72.26	72.13	72.38	71.76	69.09	72.70	69.09–76.54	72.24	2.08
Al_2O_3	14.78	11.73	14.28	14.28	15.28	14.77	17.33	14.28	11.73–17.33	14.59	1.54
FeO	0.66	0.18	0.52	0.62	0.65	0.97	0.90	0.80	0.18–0.97	0.66	0.25
Fe_2O_3	0.90	2.05	2.35	1.86	1.03	1.23	1.15	1.60	0.90–2.35	1.52	0.53
CaO	1.12	1.35	1.57	1.79	1.34	1.12	1.79	1.35	1.12–1.79	1.43	0.27
MgO	0.64	0.16	0.81	0.81	0.64	0.48	1.12	0.97	0.16–1.12	0.70	0.30
Na_2O	3.40	1.85	2.83	2.90	3.30	2.08	2.53	2.46	1.85–3.40	2.67	0.55
K_2O	5.30	4.70	3.34	4.07	3.79	6.20	4.13	4.37	3.34–6.20	4.49	0.91
TiO_2	0.84	0.18	0.41	0.25	0.26	0.17	0.21	0.30	0.17–0.84	0.33	0.21
MnO	0.21	0.01	0.06	0.04	0.04	0.01	0.01	0.02	0.01–0.21	0.05	0.06
Moist	0.17	0.40	0.18	0.24	0.17	0.13	1.05	0.16	0.13–1.05	0.31	0.30
LOI	0.38	0.12	0.40	0.50	0.39	0.27	0.36	0.20	0.12–0.50	0.33	0.12
P_2O_5	0.07	0.05	0.05	0.07	0.02	0.02	0.02	0.09	0.02–0.09	0.05	0.03
Rb	88	128	83	69	100	170	124	114	69–170	110	30
Cu	8	5	11	14	1	2	2	8	1–14	6	4
Ba	308	367	277	232	480	200	255	238	200–480	295	85
Bi	128	28	94	106	nf	6	3	102	0–128	58	51
CO	78	78	46	42	52	70	39	41	39–78	56	16
Ni	104	91	90	97	120	52	52	99	52–120	88	23
Zn	98	52	56	40	52	15	14	21	14–98	44	26
V	160	nf	30	20	60	—	—	20	0–160	48	53
Pb	18	114	27	28	52	65	59	6	6–114	46	32
Ga	37	46	31	27	38	16	58	30	16–58	35	12
Sr	354	46	527	652	507	214	106	627	106–652	379	220
Mo	nf	nf	nf	nf	14	25	nf	nf	0–25	5	9
Cr	151	83	212	138	—	—	—	111	83–212	139	43
Li	13	5	13	13	—	—	—	15	5–15	12	3

Major and minor elements in wt. %
Trace elements in ppm
nf, not found; —, not determined.

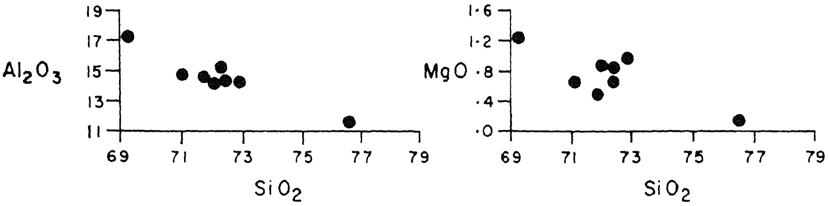

Figure 4. Harker variation diagram for MgO and Al_2O_3.

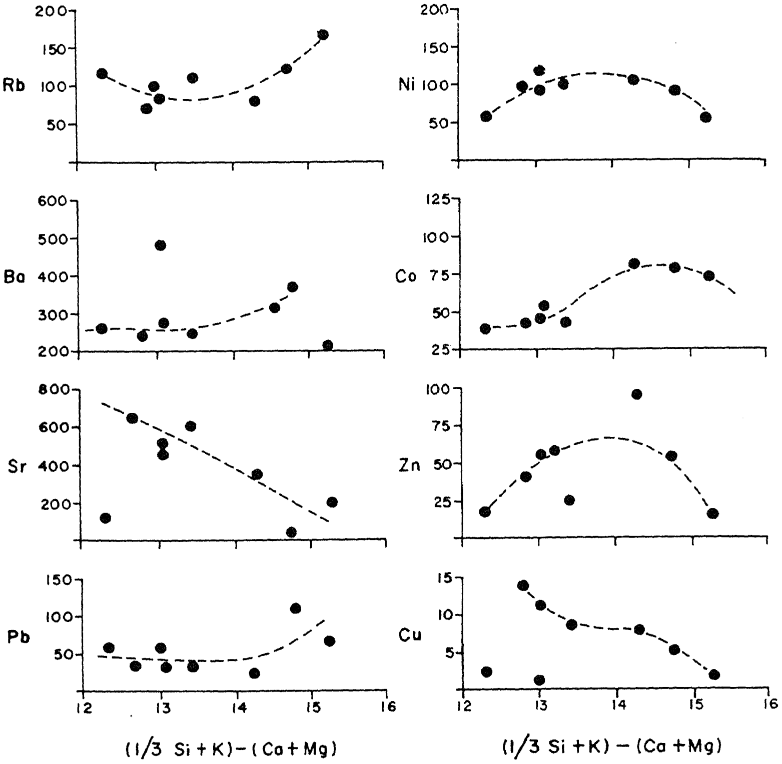

Figure 5. Variation diagrams—(1/3 Si + K) − (Ca + Mg) vs trace elements.

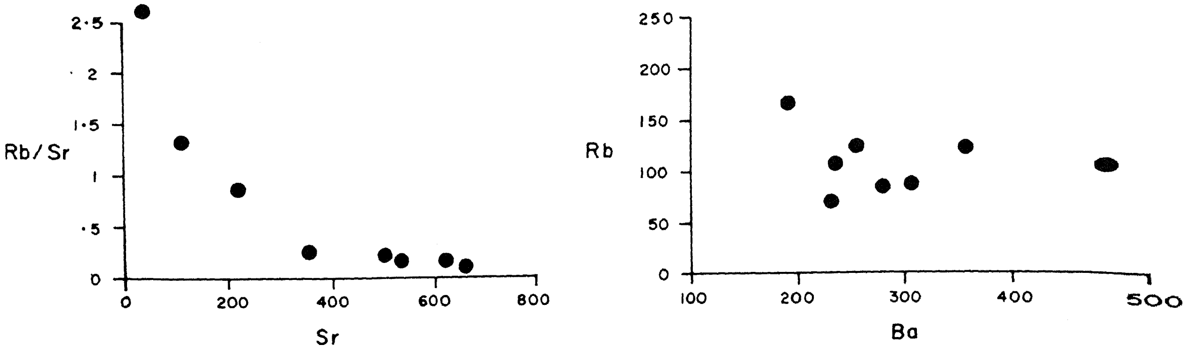

Figure 6. Rb/Sr vs Sr and Rb vs Ba plots.

of the granite range from 1.11 to 1.67 the granite could be categorized as peraluminous[14].

Harker variation diagrams in respect of Al_2O_3 and MgO are given in figure 4. Variation of trace elements is plotted against the index of differentiation (1/3 Si + K)–(Ca + Mg) (figure 5). K/Rb ratio ranges from 256 to 490 (av. 321) indicating a Rb depletion tendency. Critical trace element variation such as inter-dependence of Rb and Rb/Sr with Ba and Sr respectively (figure 6) indicates crystal fractionation

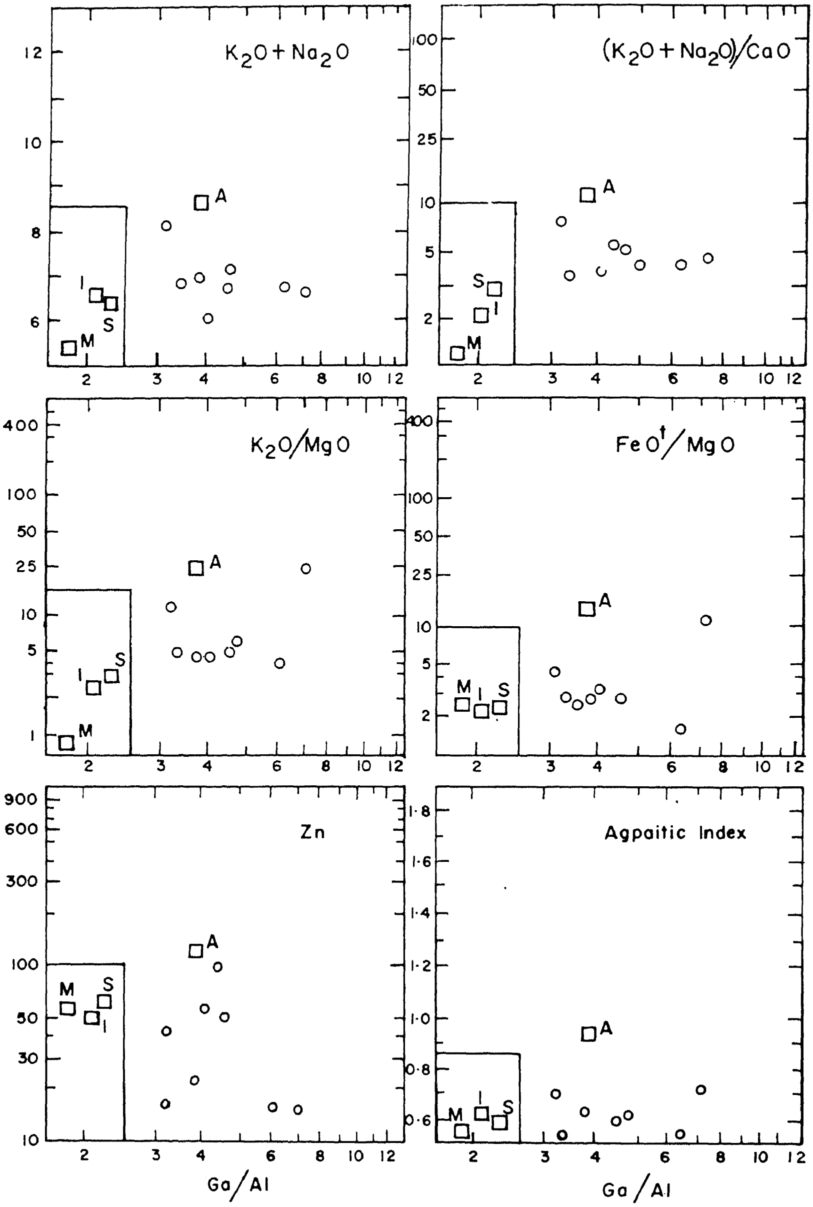

Figure 7. Ga/Al vs $K_2O + Na_2O$, $(K_2O + Na_2O)/CaO$, K_2O/MgO, FeO^t/MgO, Zn and agpiatic index plots. Coordinates indicate field of I-, S- and M-type granites. Square boxes are I-, S-, M- and A-type granite averages[26].

as an inherent process in the evolution of this granite[15].

DISCUSSION

Geochemical characters of the granite favour an igneous source. A genetic classification has been suggested for granitic rocks into those originated from sedimentary protoliths (S-type) and those evolved from igneous protoliths (I-typ)[16]. Subgroups of I-type granites are those derived from recycled, dehydrated continental crust (A-type)[17,18] and those evolved directly from subducted ocean crust or overlying mantle (M-type)[19–21]. The present granite with its moderately high SiO_2, Na_2O+K_2O, Fe/Mg, Ga/Al and low Ba and Sr is comparable with the A-type[17,18,22]. It was demonstrated that good discrimination can be obtained between A-type granites and most orogenic granites (M–I- and S-type) in plots employing Ga/Al ratios[23]. Figure 7 shows plots to Ga/Al vs K_2O+Na_2O, K_2O/MgO, $(K_2O+Na_2O)/CaO$, FeO/MgO, Zn and agpiatic index. All plots of samples clearly fall away from M–I–S-type granite field and indicate their A-type designation.

A-type granites are currently considered to represent the final plutonic event in both orogenic belts and the rift-related anorogenic magmatism of shield areas[23]. It has been suggested that the younger granite bodies of Kerala region are associated with the taphrogenesis of the continental margin[12,24,25] and many of the major lineaments in this region are rift-related[26]. Similar granite plutons in this region are correlated with the Pan-African thermotectonic regime recognized in other shield areas[12].

Among the petrogenetic models suggested, the partial melting model[18,27–30] seems to be more applicable in the present case. The granulite country rocks would have served as a viable source of dehydrated reasonably sialic crest for the generation of the magma. The attainment of the high melting temperature (> 830°C) required to partially melt this dehydrated granulitic source to generate A-type granite magma might have been achieved by the emplacement of mantle derived mafic magmas into the lower crest. The fracture lineaments which are rift-related[26] and prevalent in the region must have served as conduits to lead the magma to high level. A-type granites are economically potential source for Sn, Mo, Nb, W, Ta and F[18,20]. Further investigations are needed to confirm the economic potential of these types of granites in Kerala region.

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SHORT COMMUNICATIONS

A GENERALIZED LOGISTIC MODEL FOR ANALYSIS OF DOSE-RESPONSE DATA

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In order to study the relationship between a binary zero-one response variable and one or more explanatory variables, the logistic model has been a very useful tool because of its closeness to normal, simplicity of its form, ease of interpretation of results and its importance as a response curve. The probability density function (p.d.f.) and the corresponding cumulative distribution function (c.d.f.) of a logistic random variable **X** are given respectively by

$$f(x) = F_0(x)[1 - F_0(x)] \qquad -\infty < X < \infty, \tag{1}$$

$$F_0(x) = [1 + e^{-x}]^{-1} \qquad -\infty < X < \infty. \tag{2}$$

In particular, the logit model has many useful and practical applications in the analysis of quantal response data, epidemiologic data, dose-response curves, population growth studies and the analysis of bioassay data used by Berkson[1], Brown[2], George and Ojo[3] and others.

In this paper we embed the logit model into a more general parametric family of models, the family of generalized logistic, in which the logistic is its special case. The generalized logistic distribution is derived as follows. Let **X** be a random variable with a p.d.f. of an extreme-value distribution of exponential type given by

$$f(x|\alpha) = \frac{\alpha^n \exp(-nx) \exp(-\alpha \exp(-\alpha))}{\Gamma n}$$

$$\begin{gathered} -\infty < x < \infty \\ 0 < \alpha < \infty, \quad 0 < n < \infty. \end{gathered} \tag{3}$$

Assume that the parameter α in (3) has a gamma distribution with parameter m. Then the p.d.f. of α is given by

$$g(\alpha) = \exp(-\alpha)\alpha^{m-1}/\Gamma m. \tag{4}$$

The p.d.f. of the compounding distribution based on expressions (3) and (4) is obtained as follows:

$$\begin{aligned} f(x) &= \int_0^\infty f(x|\alpha)\, g(\alpha)\, \mathrm{d}\alpha, \\ &= \frac{\exp(-nx)[1 + \exp(-x)]^{-(m+n)}}{B(m,n)}, \\ &= \frac{[F_o(x)]^m [1 - F_o(x)]^n}{B(m,n)}, \end{aligned} \tag{5}$$

where

$F_0(x)$ is the logistic function given in (2) and $B(m,n)$ is the complete beta function with

$$(m+n-1)B(m,n) = (n-1)B(m,n-1). \tag{6}$$

Using (5) the c.d.f. of **X** is given by

$$F(x) = \frac{1}{B(m,n)} \int_0^{F_0(x)} w^{m-1}(1-w)^{n-1}\mathrm{d}w. \tag{7}$$

We call $F(x)$ the generalized logistic distribution with parameters m and n and denote it by $GL(m,n)$. This family of the standard logistic is quite rich and contains many of the well-known distributions as its special or limiting distributions. For example, if $m=n=1$, (7) becomes the logistic distribution; if $(m,n) \to (\infty, \infty)$, the $GL(m,n)$ converges to the normal distribution; it converges to an extreme minimum value if $(m,n) \to (1, \infty)$ and to an extreme maximum value if $(m,n) \to (\infty, 1)$; it also converges to a double exponential if $(m,n) \to (0,0)$, to an exponential if $m \neq 0$ and $n \to 0$ or if $m \to 0$ and $n \neq 0$. It should also be noticed here that $GL(m,n)$ is symmetric if $m=n$, positively skewed if $m>n$ and negatively skewed if $m<n$.

The three forms of the standard generalized logistic derived by Balakrishan and Leung[4] are the special cases of our generalization. For $n=1$, (7) becomes $GL(m,1)$ and is given by

$$F(x) = [F_0(x)]^m \tag{8}$$

and p.d.f. of **X** as

$$f(x) = m[F_0(x)]^m [1 - F_0(x)]. \tag{9}$$

$GL(m,1)$ is a family of positively skewed distributions with its coefficient of kurtosis greater than that of the logistic when $m>1$. For $m=1$, (7) is $GL(1,n)$ and is given by

$$F(x) = 1 - [1 - F_0(x)]^n. \tag{10}$$

Dose-response data;
Generalized logistic distribution

Table 1 *Mortality of adult flour beetles after 5 h exposure to gaseous carbon disulphide*

CS_2 mg/l	Dosage in $\log_{10} CS_2$ mg/l	Number of insects exposed		Number of insects killed	
		Data set I	Data set II	Data set I	Data set II
49.06	1.6907	29	30	2	4
52.99	1.7242	30	30	7	6
56.91	1.7552	28	24	9	9
60.84	1.7842	27	29	14	14
64.76	1.8113	30	33	23	29
68.69	1.8369	31	28	29	27
72.61	1.8610	30	32	29	32
76.54	1.8839	29	31	29	31

It is a family of negatively skewed distributions with its coefficient of kurtosis greater than that of the logistic when $n > 1$. For $m = n = P$, (7) is $GL(P, P)$ and is given by

$$F(x) = \frac{1}{B(P,P)} \int_0^{F_0(x)} [w(1-w)]^{P-1}\, dw. \qquad (11)$$

It is a family of symmetric distributions with a coefficient of kurtosis smaller than that of the logistic and decreasing with increasing values of P. This family of distributions is very close to the normal distribution.

We expect that this family of generalized logistic, in which the logistic and probit models are special cases, should provide a better fit. In the next section, we illustrate the application of the generalized logistic model, $GL(m, 1)$, to two data sets derived from the experiments of Strand[5] with Tribolium confusum.

Applications to dose-response data

The mortality of the adult flour beetles after 5 h exposure to known concentrations of gaseous carbon disulphide for two experimental series, designated as I and II, selected from the experiments of Strand[5] is given in table 1. A plot of percentage killed, in each data set, against dosage suggests that the dose-response curve may be skewed. Therefore the generalized logistic $GL(m, 1)$ model is considered a good candidate to fit these data sets.

For comparison we also fit the logit model, and the results of the probit analysis obtained by Bliss[6] are included. The following three models are used:

Logit model

$$F_0(\alpha + \beta x_i) = \{1 + \exp[-(\alpha + \beta x_i)]^{-1}\}.$$

GL(m, 1) model

$$F(\alpha + \beta x_i) = [F_0(\alpha + \beta x_i)]^{-m}.$$

Probit model

$$F(\alpha + \beta x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\alpha + \beta x} \exp(-z^2/2)\, dz,$$

where α, β and m are unknown parameters to be estimated from the observed data and x_i is the ith dosage (in $\log_{10} CS_2$).

Analysis of data set I: The logit model fit yields $\hat{\alpha} = -60.6156$ and $\hat{\beta} = 34.2129$, while the $GL(m, 1)$ model provides $\hat{\alpha} = -107.90$, $\hat{\beta} = 59.43$ and $\hat{m} = 0.3081$. Bliss[6] treated the data by fitting separate probit models to the lowest two concentrations yielding $\hat{\alpha} = -15.4336$, $\hat{\beta} = 11.35$, and for the highest 3rd–8th concentrations the estimates are $\hat{\alpha} = -40.3838$ and $\hat{\beta} = 25.51$.

Analysis of data set II: The maximum likelihood estimates of the unknown parameters obtained by the logit model are $\hat{\alpha} = -66.6508$ and $\hat{\beta} = 37.6683$. The $GL(m, 1)$ model fit yields $\hat{\alpha} = -195.60$, $\hat{\beta} = 107.60$ and $\hat{m} = 0.1695$. Bliss's estimates for the lowest two concentrations are $\hat{\alpha} = -15.1941$, $\hat{\beta} = 11.35$, and for the highest six concentrations are $\hat{\alpha} = -39.7413$ and $\hat{\beta} = 25.15$.

The expected number of insects killed, computed

Table 2 *Comparison of observed and expected mortality of adult flour beetles (data set I)*

Obs number killed	Exp number killed Logit	Probit	$GL(0.3081, 1.0)$
2	1.7072	3.1900	2.9133
7	4.9327	5.8140	5.5589
9	10.1465	7.5992	9.0901
14	16.3399	14.9094	14.3968
23	23.8448	23.8380	23.0922
29	27.9906	28.8300	28.6452
29	28.6495	29.4510	29.3829
29	28.3887	28.8985	28.8420

Table 3 *Comparison of observed and expected mortality of adult flour beetles (data set II)*

Obs number killed	Logit	Exp number killed Probit	*GL* (0.1695, 1.0)
4	1.4709	4.7310	2.9511
6	4.6217	8.0130	5.4372
9	12.5547	6.5976	10.8452
14	18.4368	16.0138	15.6331
29	27.3534	26.1327	27.3642
27	25.9571	25.9672	27.4325
32	31.0153	31.3728	31.9482
31	30.5901	30.8698	30.9957

by the three models as well as the observed number killed, are given in tables 2 and 3 for data sets I and II, respectively. Table 2 suggests that the *GL* (0.3081, 1.0) model is a more appropriate response function for data set I. On the other hand, table 3 shows that the *GL* (0.1695, 1.0) model fits data set II much better compared to the logit and probit models.

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NEW RECORDS OF FUNGI FROM INDIA

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DURING studies on curd-rot and its relationship with aeromycoflora at Agra, several fungal species were isolated, out of which the two species briefly described below were found to be new records for India.

Phoma medicaginis Malbr. & Roum. (original reference—*Rev. Mycologique Toulouse*, 1886, **8**, 91): Isolated from the aeromycoflora of a cauliflower field at Agra (Near Namner) in July 1986. Colonies on Czapek's agar dark-brown to black; hyphae septate, branched, blackish-brown; pycnidia developing in 15-day-old cultures, numerous, round to ovoid, black, ostiolated, 57–104 μm × 80–172 μm; conidia hyaline, single-celled, ovoid, 7 × 5 μm. Specimen deposited at CAB International Mycological Institute, Kew, England (IMI 313255).

Tritirachium oryzae (Vincens) de Hogg. (original reference—*CBS Studies in Mycology*, 1972, **1**, 22): Isolated from the aeromycoflora of cauliflower field at Agra (Near Namner) in October 1986. Colonies of Czapek's agar whitish, restricted, actinomycetous type, reverse pale yellowish; mycelium hyaline, tortuous, septate, sparingly branched, branches usually appearing near septa; conidiophore upright, long, slender, triverticillately to pentaverticillately branched; sporogenous branches tapering, 42–70 μm in length, fertile portion zig-zag; conidia apical on sympodially formed growing region, hyaline, unicelled, globose to ovate, 1.25–2.0 μm × 2.0–2.5 μm. Specimen deposited at CAB International Mycological Institute, Kew, England (IMI 319328).

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HERMAPHRODITE FLOWERS IN DIOECIOUS *MOMORDICA DIOICA* ROXB.

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MOMORDICA DIOICA is a semi-wild, perennial, tuberous and distinctly dioecious species of the Cucurbitaceae[1,2]. Polyploidization was induced in this species by colchicine treatment. Artificial triploids were also raised by crossing the diploid female with induced tetraploid male and reciprocal crosses[3]. All the cytotypes (diploid, triploid and tetraploid) were dioecious. But one of the hybrid males showed monoecious character and rare occurrence of hermaphrodite flowers on the branch bearing female flowers. This communication deals with morphology and pollen behaviour of hermaphrodite flowers.

Seeds of the hybrid between tetraploid female and diploid male were sown. During the first season one plant in the F$_1$ generation grew vigorously and produced only male flowers. In the following season the same tuber produced some female twigs and

Cytotypes

bore many female flowers like those of normal hybrid triploid female plants. But the sex expression changed from a strictly dioecious to monoecious. However, one female branch of the monoecious hybrid triploid produced several hermaphrodite flowers. The flowers were smaller in size and deep yellow in colour like those of diploid plants. The number of petals in the hermaphrodite flowers varied from 6 to 8. This number is always 5 in male and female triploid plants. The number of stamens in the hermaphrodite flowers varied between 1 and 3, whereas it is always 3 in the dioecious triploid male. No fruit setting occurred in the bisexual flowers. When pollen grains from the hermaphrodite flowers were dusted on diploid, triploid and tetraploid female flowers, no fruit formation took place. Pollen grains, when examined in acetocarmine, were found to be empty.

The report of hermaphrodite flowers on a dioecious species is an example of reversal of sex type to that of the bisexual progenitors. In some other cucurbits such as *Coccinia indica*[4] and *Momordica charantia*[5] also cases of this type have been recorded. This suggests that the bisexual type is the most primitive, from which monoecious and dioecious types have gradually evolved.

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EFFECT OF SODIUM CHLORIDE ON IONIC COMPOSITION OF *AZOLLA PINNATA*

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AZOLLA PINNATA has been used as nitrogen fertilizer for rice. Experimental work on *A. pinnata* has revealed that this water fern possesses the capacity to absorb and withstand sodium chloride in its system. This property of *Azolla* could be profitably employed for reclaiming salt-affected lands which are encroaching upon good agricultural lands and rendering the latter unfit for crop cultivation[1]. It was therefore of interest to study the effects of sodium chloride on the ionic composition of *A. pinnata* and to determine the maximum levels of sodium chloride that it could tolerate.

Experiments were conducted on aseptically cultured *Azolla pinnata* R.Br. in nitrogen-free medium[2]. *Azolla* (300 ± 20 mg) was subcultured in nitrogen-free medium (30 ml, control) and in medium (30 ml) supplemented with 5, 10, 20, 30, 40 and 50 mM sodium chloride. The pH of the medium was adjusted to 5.6 before autoclaving. Cultures were incubated at 25 ± 2°C in white fluorescent light (1000 lux), 16/8 h photoperiod, with weekly renewal of culture medium.

Azolla samples from each treatment were harvested and dried separately after three weeks of culture. Dried samples were ashed in a nickel crucible in a muffle furnace for 2 h at 500°C. Copper stubs with double-sided taps were pressed firmly into each ash sample and mineral composition of the ash was determined[3]. The analysis was carried out using a scanning electron microscope (Philips) provided with a computerized energy dispersive X-ray system.

Table 1 shows that *A. pinnata* cultured in nitrogen-free medium (control) contained maximum amounts of calcium, potassium and magnesium compared with samples cultured in sodium chloride-containing medium. In *Azolla* treated with sodium chloride, there was a linear increase in sodium and chloride, with a progressive decrease in biomass. At 40 mM sodium chloride in the culture medium, sodium and chloride were higher by 77% and 151% respectively compared with the control. This may be due to uptake and accumulation of sodium and chloride ions by *Azolla* from the medium. This is similar to the behaviour of other glycophytes[4] and sorghum[5].

It was observed that *Azolla* could grow in

Table 1 *Biomass and ionic composition of Azolla pinnata cultured in nitrogen-free medium supplemented with various levels of sodium chloride*

NaCl level (mM)	Fresh weight (mg)	Dry weight (mg)	Sodium (mg/g dry wt)	Chloride (mg/g dry wt)	Calcium (mg/g dry wt)	Potassium (mg/g dry wt)	Magnesium (mg/g dry wt)
Control (NaCl absent)	2590	103	4.3	7.9	8.9	29.8	4.9
5	2570	102	4.9	8.6	8.6	29.3	4.9
10	2530	101	5.1	9.1	8.5	29.0	4.9
20	1760	72	5.6	10.7	7.3	26.1	4.9
30	1080	39	6.3	15.3	6.1	19.8	4.7
40	720	29	7.6	19.8	5.3	11.4	4.5
50	—	—	—	—	—	—	—
CD at 5%	134	7.3	—	—	—	—	—

medium containing up to 40 mM sodium chloride indicating that this level is tolerable, but further increase did not allow growth.

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BIOCHEMICAL CHANGES IN TUKRA-AFFECTED EXOTIC MULBERRY PLANTS

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MEALYBUGS causing tukra disease[1] in mulberry were reported by Roy[2] and Paul[3] in 1941. The bugs feed on the tender leaves and shoots of mulberry. The affected plants show curling of leaves at the growing point and arrested growth. The disease is caused by a virus transmitted by the common mealybug, viz. *Maconellicoccus hirsutus*[4,5]. Rearing young-age silkworms is solely dependent on the tender leaves of mulberry.

In tukra the leaves are very badly damaged. Not only do the leaves show morphological changes (deformed, crinkled, wrinkled, etc.), but biochemically also they differ[6,7] from normal leaves. This affects the nutritional status of the leaves. During a survey, between August 1985 and February 1986, of mulberry plants in the germplasm bank in the Bangalore University campus, *Morus australis*, *M. cathyana*, *M. macroura* and *M. nigra* were reported to be free from tukra attack[8]. However, in May–June 1988, these plants were also severely affected in the same garden as all the other varieties were also attacked by the mealybugs. Biochemical changes in leaves of the four exotic species mentioned above were investigated.

Tukra-affected and unaffected leaf samples of *M. australis* Pair. (Australia, Indonesia, Philippines, South China), *M. cathyana* Hemsl. (Central China), *M. macroura* Mig. (China, Indonesia, Japan) and *M. nigra* L. (Persia, West Asia) were collected during May–June 1988 and oven-dried. The samples were analysed for total chlorophyll[9], protein[10], sugars[11], reducing sugars[12], phenols[13] and starch[14].

The data are given in table 1. There was a decrease in protein content of *Morus macroura* and *M. nigra* affected by tukra; the reduction was, however, negligible in *M. nigra*. Tukra caused an increase in protein content of the other two species, viz. *M. australis* and *M. cathyana*. In an earlier study[7] protein content decreased by 10.5% in mulberry variety Kajali while in variety Kanva-2 it increased significantly (40%). Increase in protein content may be due to induced synthesis of protein in response to the infection[15], while reduction in protein content may be attributed to hydrolysis by proteolytic enzymes secreted by the pathogen[16].

Morus macroura and *M. nigra* affected by tukra showed a decrease in the level of phenols. On the

Table 1 *Biochemical changes in tukra-affected exotic mulberry species*

Species		Protein	Phenols (all mg/g dry wt)	Starch	Reducing sugars	Sugars
Morus australis	Uninfected (UI)	142.50	90.00	109.80	48.00	132.00
	Infected (I)	162.00	118.00	106.20	49.50	128.00
	Change (%)	+13.68	+31.11	−3.27	+3.12	−3.30
M. cathyana	UI	115.40	95.00	99.00	42.00	120.00
	I	126.00	115.00	97.20	37.60	118.00
	%	+9.18	+21.05	−1.80	−10.47	−1.66
M. macroura	UI	136.85	128.00	73.80	44.00	92.00
	I	128.50	110.00	93.15	51.50	113.50
	%	−6.10	−14.06	+26.21	+17.04	+23.36
M. nigra	UI	130.50	125.00	113.20	30.00	158.00
	I	127.60	115.00	127.08	28.00	151.20
	%	−2.22	−8.00	+12.28	−6.66	−4.30

Each value is mean of six samples.

contrary, there was a significant increase in *M. australis* and *M. cathyana*. Such increase or decrease in phenolic content has been noticed in a number of other host–pathogen combinations[17, 18]. Interaction between the pathogen and the host might have triggered production of more phenols. Decrease in phenolics may be a result of severe infection[19].

Starch content was, however, increased in tukra-affected *M. macroura* and *M. nigra*. The increase was significant in *M. macroura*. There was only negligible decrease in *M. australis* and *M. cathyana*. In the earlier study[7] Kajali and Kanva-2 showed an increase in starch content. Alabi and Nagvi[20] suggested that starch is not normally utilized for growth by the pathogen, hence it accumulates in the leaf tissue. Reduction in the starch content of affected leaves may be due to enzymatic activity of the pathogen, as evidenced by the increased reducing sugar content of infected parts[21].

Sugar content of tukra-affected *M. australis*, *M. cathyana* and *M. nigra* was marginally decreased. However, tukra caused significant increase in sugar and reducing sugar level of *M. macroura*. An increase in reducing sugar was noticed in tukra-affected *M. australis* also, but it was negligible. In *M. cathyana* and *M. nigra*, tukra brought down the level of reducing sugars. The two indigenous mulberry varieties, Kajali and Kanva-2, also showed an increase in sugar content when affected by tukra[7]. It also appears that the infection induces production of soluble sugars, which may be metabolized by the pathogen for its growth[22]. Decrease in reducing sugars may be due to the reduction in leaf lamina and malformation of leaves in tukra-affected plants. A close study of the changes in reducing sugars and phenols in infected plants showed that phenols accumulated at the expense of reducing sugars in all the species except *M. macroura*. This is in conformity with the work of Rama Pandu and Raychoudhuri[23]. However, the overall decrease in reducing sugars could be due to the utilization of the sugars by the pathogen for its multiplication. Increase in reducing sugar content after infection may be due to disturbed host metabolism and marked stimulation of CO_2 fixation in the affected leaves[24].

Total chlorophyll, chlorophyll *a* and *b* and chlorophyll *a*/*b* ratio in healthy and tukra-affected plants are given in table 2. There was significant reduction in total chlorophyll, chlorophyll *a* and *b* and chlorophyll *a*/*b* ratio in tukra-affected *Morus cathyana*. Although *M. australis*, *m. macroura* and *M. nigra* recorded an increase in total chlorophyll and chlorophyll *a* and *b*, the *a*/*b* ratio was decreased. Increase in total chlorophyll and chlorophyll *a* and *b* was significant in *M. nigra*, marginal in *M. macroura* and slightly more in *M. australis*. Except chlorophyll *b* in variety Kajali, all these quantities were increased as a result of tukra attack in both Kanva-2 and Kajali varieties[7].

Diseased tissue usually contains considerably less chlorophyll than healthy leaves. It is interesting that chlorophyll content, particularly chlorophyll *b*, was higher in tukra-affected plants, except *M. cathyana*.

Increase in chlorophyll content of tukra-affected leaves may be due to failure of the pathogen to inhibit chlorophyllase enzymes, as in okra[24]. Roberts and Corbett[25] reported reduced photosynthesis in

Table 2 *Changes in chlorophyll content of tukra-affected exotic mulberry species*

Species		Chlorophyll (mg/g dry wt)			
		Total	Chl *a*	Chl *b*	*a/b* Ratio
Morus australis	Uninfected (UI)	4.82	2.60	2.25	1.155
	Infected (I)	5.14	2.72	2.42	1.127
	Change (%)	+6.63	+4.61	+7.55	−2.424
M. cathyana	UI	3.91	2.05	1.86	1.102
	I	3.03	1.45	1.58	0.917
	%	−22.50	−29.26	−15.05	−16.787
M. macroura	UI	5.28	2.72	2.56	1.062
	I	5.52	2.84	2.68	1.059
	%	+4.54	+4.41	+4.68	−0.282
M. nigra	UI	4.50	2.48	2.02	1.227
	I	5.20	2.85	2.35	1.212
	%	+15.55	+14.91	+16.33	−1.220

Each value is mean of six samples.

tobacco plants and demonstrated that a symptomless tissue of low virus content showed reduced photosynthesis per unit of chlorophyll, suggesting that perturbation of photosynthesis did not result from chlorophyll loss; on the contrary, the tissue had slightly increased chlorophyll content per unit leaf area and appeared greener than uninfected tissue. Similar results were obtained in Kanva-2 variety of mulberry in powdery mildew and virus infections[26].

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SCANNING ELECTRON MICROSCOPIC OBSERVATIONS OF *DIASCHISTORCHIS GASTRICUS*

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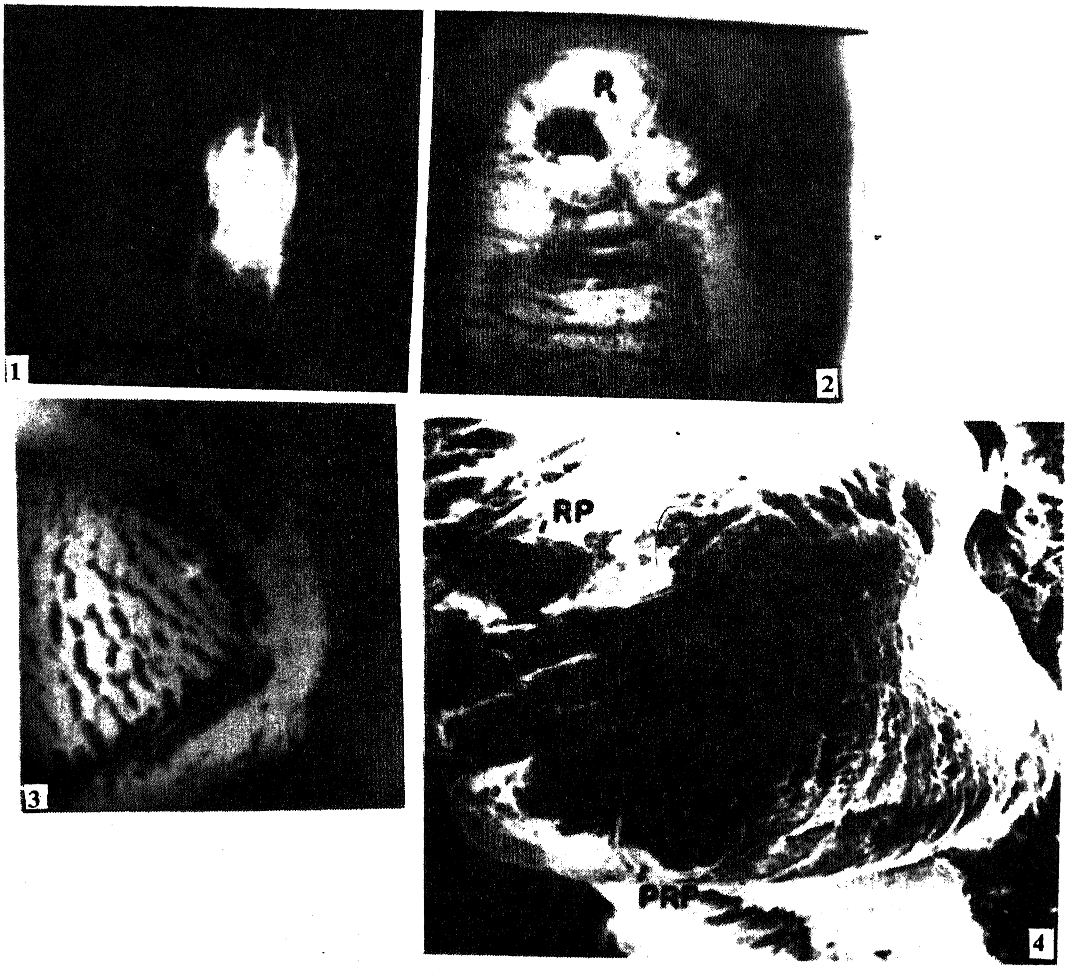
THE surface ultrastructure of trematodes has attracted the attention of many workers. Some of the notable contributions are on *Leucochloridium* sp.[1], *Urogonimus macrostomum*[2], *Phyllodistomum*[3], *Philopthalmus megalurus*[4], *Megalodicus temperatus*[5], *Gorgoderina attenuata*[6], *Isoparorchis hypselobagri*[7] and *Encyclometra colubrimurorum*[8].

The different species of schistosomes examined by scanning electron microscopy (SEM) are *Schistosoma mansoni*[9], *S. haematobium*[10], *S. intercalatum*[11], *S. japonicum*[12], *S. spindalis* and *S. nasalis*[13].

The present communication is based on a scanning electron microscopic study of *Diaschistorchis gastricus*, a monostome parasite in the stomach of the freshwater turtle *Kachuga tactum tentorium*.

D. gastricus was collected from the stomach of *K. tactum tentorium*. After collection, the parasites



Figures 1–4. Surface features of *Diaschistorchis gastricus* revealed by SEM. **1,** Dorsal surface, showing folds. **2,** Region around the oral sucker, showing papillae (P). **3,** Ventral surface, showing numerous grooves and ridges. **4,** Posterior end, showing retracted (RP) and protracted (PRP) papillae.

were washed well in cold Tyrode's solution. Worms were fixed in 3% glutaraldehyde buffered with cacodylate (pH 7.4) for 2 h, followed by post-fixation in 1% osmium tetroxide, and dehydration through a series of alcohol. The specimens were mounted on brass stubs in various positions using silver paint and coated with approximately 200 Å of gold in a vacuum evaporator. They were examined in a JEOL JSM-35 scanning electron microscope operating at 10–15 kV.

SEM studies of surface features of monostome trematodes are scanty. The other monostomes studied are *Notocotylus attenuatus*[14], *N. triserialis*[15].

In *D. gastricus* the dorsal surface is convex and has uniform folds (figure 1). The transversely oriented ridges and grooves of the dorsal surface are highly convoluted as in *Isoparorchis hypselobagri*[7]. These ridges become narrow towards the lateral regions.

The ventral surface is concave. The oral sucker is distinct from the rest of the ventral surface and the oral aperture is guarded by radiating ridges and grooves as in *L. constantinae*[16] and *Cotylogaster occidentalis*[17].

A few aciliated blunt papillae are seen on the lateral region of the oral sucker (figure 2). Such sensory papillae were also reported in *L. constantinae*[16].

The posterior truncated margin is also ventrally curved, thus making the ventral surface boat-shaped. The ventral surface is made up of transverse grooves and ridges which are highly rugose without any papillations or spines, giving a spongy look (figure 3). The ridges on the margins are narrower than those on the concave median ventral surface.

The truncated posterior end has a pair of conical protuberances or papillae at their lateral corners. Some of these papillae were in retracted condition and some in protracted condition (figure 4).

The oral sucker is well developed and distinct from the rest of the ventral surface, with prominent radiating ridges and grooves. These may help in continuous contraction and expansion, facilitating adhesion and feeding. The sensory papillae near the margin of the oral sucker may be chemoreceptors. The general body surface of both the dorsal and ventral surface is thrown into transverse bands of ridges and grooves which are rugose. This wrinkling of the body surface may be for increasing the surface area of absorption.

Live worms were seen to be firmly attached to the mucosa of the stomach wall. It was not easy to dislodge them from the host tissue. The entire length of the worm was firmly fixed along the ventrolateral and posterior margins. In the authors' opinion the method of attachment of the worms is to make the bodies act as suction cups as in *N. triseriatis*[15].

In *D. gastricus*, the lateral edges and the conical posterolateral papillae are highly mobile. In the fixed and coated specimens observed by SEM, some papillae were protracted and some retracted. Radlett[14] suggested that the ventral papillae of *N. attenuatus* were used for adhesion to the host caecal mucosa. Mackinnon[15] suggested that the ventral papillae of *N. triserialis* may be involved in nutrient absorption. Wittrock[18] observed excretory ducts in the papillae of *Quinqueserialis*. In *D. gastricus* the exact function of the papillae is still unclear.

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SUSCEPTIBILITY OF CABBAGE DIAMONDBACK MOTH *PLUTELLA XYLOSTELLA* L. TO THE ENTOMOFUNGAL PATHOGEN *VERTICILLIUM LECANII* (*ZIMMERM.*) VIEGAS

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Plutella xylostella, commonly called diamondback moth of cabbage, is a serious pest on cruciferous crops like cabbage, cauliflower, knol-khol, radish, etc. The larval stage of the insect causes extensive damage to the leaf primordium especially in cabbage and cauliflower and thereby causes economic loss. A search for entomofungal pathogens of this pest did not yield any pathogen. Hence the susceptibility of the pest to the entomofungal pathogen *Verticillium lecanii* was tested. This pathogen is known to be non-specific, causing diseases in a number of insects of the orders Homoptera, Coleoptera and Lepidoptera. It is also known to infect the coffee green bug *Coccus viridis* (Green) in several countries[1].

The entomofungal pathogen *V. lecanii* isolated from the apple leaf folder *Archips pomivora* was isolated in pure culture on Sabouraud dextrose agar media. Based on the morphological characters of the conidiophore and the conidia the fungus was primarily identified as *V. lecanii* and later confirmed through CMI, London (Herb. IMI Number 327752).

The fungal spore suspension used was prepared from spores isolated from cultures derived originally from a diseased larva of *A. pomivora* collected on apple in Himachal Pradesh, India. The spores were inoculated onto sorghum grains previously soaked in water for 24 h and autoclaved at 15 psi for 30 min, as these grains were found to be suitable for culturing *C. lecanii*[2]. The fungus grew well on sorghum grains by producing clear white mycelial growth at 25 ± 2°C. Profuse sporulation was observed on the fourth day after inoculation. The spores were collected on the tenth day by using sterile distilled water with 0.01% Triton X-100. The spore suspension was filtered through two layers of muslin and the filtrate was centrifuged at 500 rpm for 10 min. The spores were washed three times thoroughly with sterile distilled water and the suspension was made in sterile distilled water containing 0.01% Triton X-100. The concentration of the spore suspension was adjusted to 2.8×10^9 spores/ml using an improved Neubauer haemocytometer. The spore suspension was used within 24 h of harvest of spores.

P. xylostella larvae were collected from a cabbage field. From the size of the head capsule, larvae of different stages (instar I to IV) were identified and sorted. Larvae looking sluggish or suspected to be parasitized by *Apanteles* sp. were discarded and only healthy larvae were used. About 24 I instar, 16 II instar, 32 III instar and 68 IV instar larvae were used.

Cabbage leaves were washed thoroughly in running water and then in sterile distilled water. One leaf was placed in each of several plastic containers (12.5 × 10.0 cm) containing moist Whatman filter paper No. 41 to provide humidity. To prevent desiccation of the leaves, the petiole was covered with a moist cotton swab. The larvae, grouped by instar, were transferred to the containers and sprayed with spore suspension using an atomizer. The containers were covered with brass wire mesh lids to provide aeration. The experiment was carried out under laboratory conditions (25 ± 2°C). Observations were made daily and mortality of the larvae was recorded.

The results are presented in table 1. The fungus (2.8×10^9 spores/ml) caused 100% mortality in young larvae (instars I and II) in 4 days. None of the IV instar larvae treated died in the larval stage, but there was some mortality in the prepupal and pupal stages.

The dead larvae become hard and mummified. White mycelial growth on the body surface between segments (figure 1) was noticed on the fifth day after inoculation and sporulation was observed on the sixth day. Prepupae and pupae from larvae that escaped infection showed mortality on the fourth and fifth day respectively after inoculation.

It is obvious that, though the fungus was originally isolated from *A. pomivora*, it also infects *P. xylostella*. About ten species of sucking pests[3] and the brinjal leaf beetle *Henosepilachna vigintioctopunctata* (Fabr.)[4] were found to be susceptible to *V. lecanii*. *V. lecanii*

Table 1 *Susceptibility of Plutella xylostella to the entomopathogenic fungus Verticillium lecanii*

Instar	No. treated	Days	Mortality (%)
I	24	4	100.00
II	16	4	100.00
III	32	4	37.50
IV	68	4–7	52.94

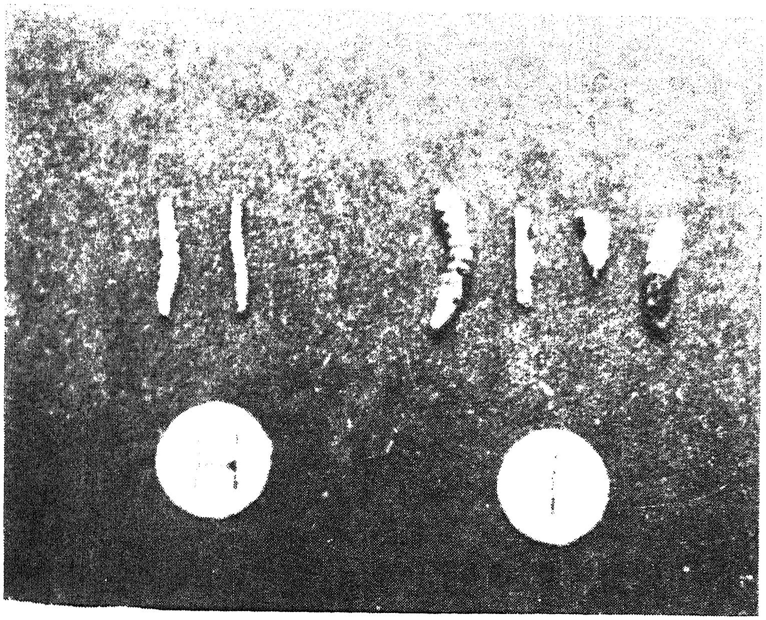

Figure 1. Healthy and *Verticillium lecanii* infected larvae of *Plutella xylostella*.

isolated from soil caused 53% mortality in white grubs *Holotrichia consaginnea* Bianch (I and II instars) in a pot culture study[5]. Thus, under suitable environmental conditions, *V. lecanii* might serve as an effective biocontrol agent against *P. xylostella* on cruciferous crops. Further studies on the efficacy of the fungus on *P. xylostella* on cabbage under glass house and field conditions are in progress.

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17 December 1988

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A NEW FRUIT ROT OF *CITRUS RETICULATA* BLANCO

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DURING surveys conducted in January–February 1986, a new fungal fruit rot of orange (*Citrus reticulata* Blanco) was observed in the orange orchards of Kolasib town of Mizoram state. In the initial stage of infection, the fruit developed minute depressions and one to several circular ash-grey spots. On ripening and subsequently, the spots turned into bigger lesions. The infected fruit coat shrivelled and gave a dry rot appearance. Black pycnidial bodies developed on these lesions (figure 1).

Microscopic examination of the isolated fungus revealed that it is *Phoma citri* (culture no. MZ. ICAR–PP–27). Whitish, greenish-dark to black colonies appeared on PDA slants. So far in Mizoram, no record of this fruit rot disease has been made on orange fruits and the presently reported host is a new record for Mizoram and India[1].

The pathogenicity test was carried out successfully by inoculating the isolate grown on PDA slants into orange fruits by Granger and Horne's method[2] and also by spraying the conidial suspension of the

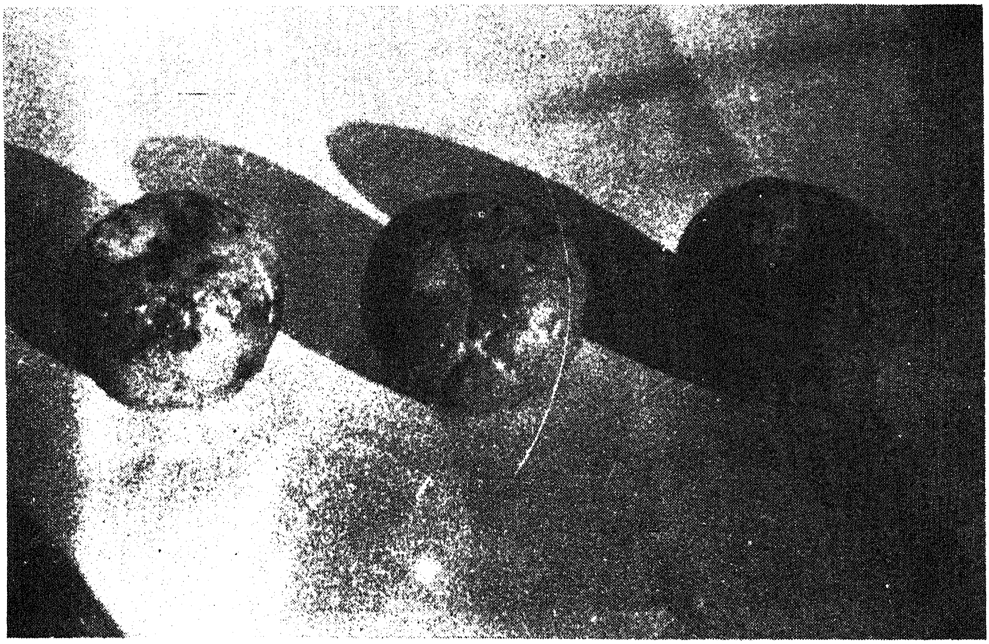

Figure 1. Orange fruits showing symptoms of *Phoma citri* infection.

organism over injured and uninjured fruits. Only injured and ripened fruits developed typical symptoms after 15 days. Reisolation from artificially infected fruits yielded pure culture of *Phoma citri*. (Culture deposited in the division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.)

Thanks are due to Dr A. K. Sarbhoy, Scientist and Curator, Mycology and Plant Pathology Division, IARI, New Delhi, for identification of the fungus.

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OUTBREAK OF *ORGYIA POSTICA* WALKER (LYMANTRIIDAE; LEPIDOPTERA), A NEW PEST ON MANGO IN UTTAR PRADESH

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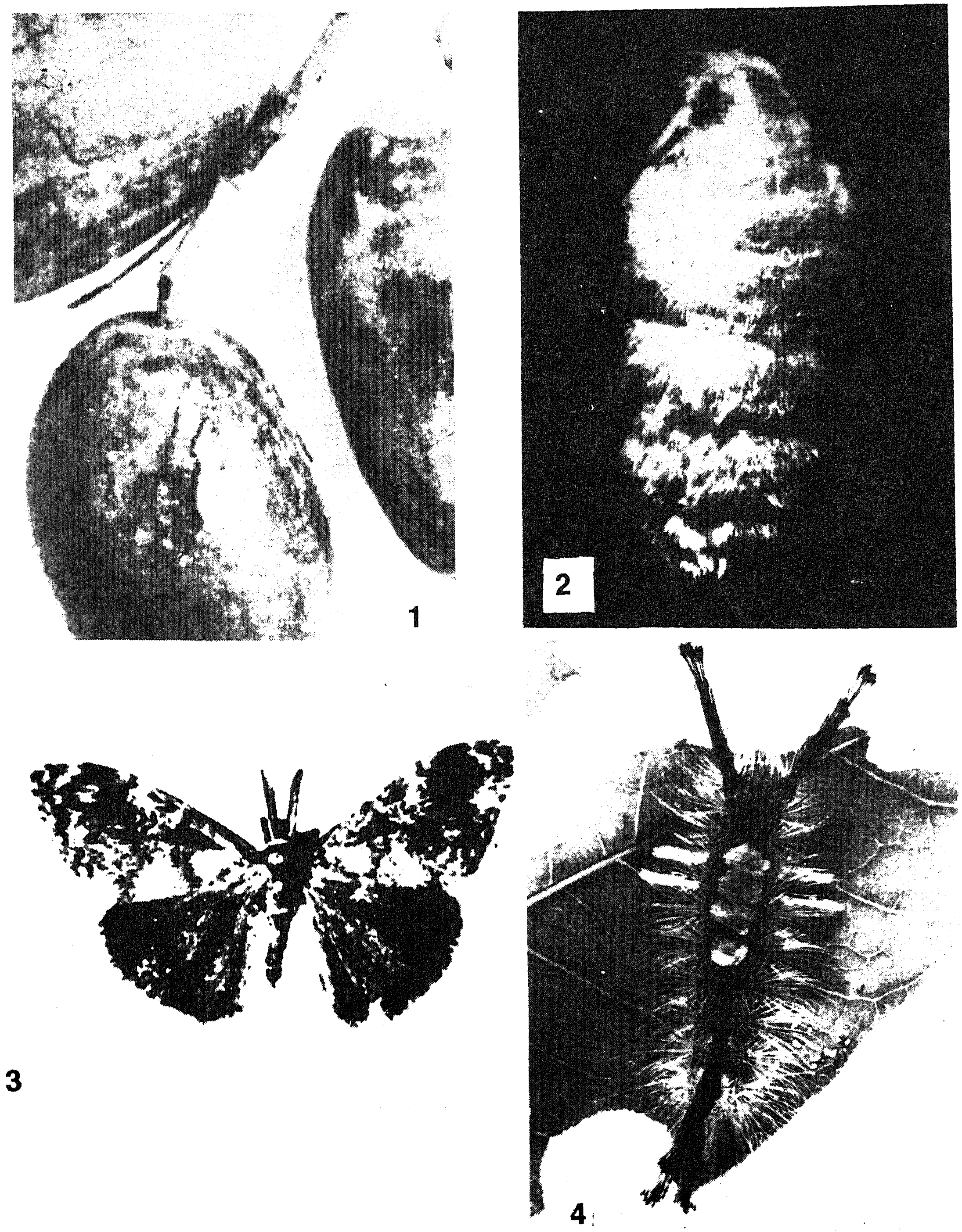
LARGE-SCALE defoliation of mango by *Orgyia postica* was noticed in orchards of Behat area in Saharanpur district (Uttar Pradesh) during June–July, 1987. Light infestation was also noticed in most of the orchards surveyed in Lucknow. Perusal of the literature revealed that this is the first record of the pest on mango from India. Host range, nature of damage, and some observations on the biology of the pest are reported here.

O. postica is a polyphagous pest attacking *Albizzia* spp., *Anogeissus latifolia, Callicarpa lanata, Erythrina lithosperma, Lagerstoemia flos-reginae, L. indica, Pithecolobium dulce, Ricinus communis, Shorea robusta, Tectona grandis, Ziziphus jujuba* and *Z. xylopyra* in India[1]. Sporadic outbreaks of this pest have been recorded on *Ricinus communis*[2]. Although a pest of broad-leaved species, it has been

Defoliation



Figures 1–4. *Orgyia postica* Walker. **1,** Damaged mango fruit and panicle; **2,** female moth; **3,** male moth; **4,** larva.

found defoliating pines and is considered a potential pest of tropical pines in India[3]. On mango it is recorded from the Philippines[4]. In addition, the authors observed the pest feeding on *Eugenia jambolana* and pear.

The young larvae feed on new flush, also nibbling the leaf and shoot buds and affecting growing points. The later stages feed on leaves and nibble new soft shoots. In case of severe attack, fruit stalks and fruits were also scraped (figure 1), resulting in drying up of affected tissues and rendering the fruits unmarketable.

The female moth, often with under-developed wings or wingless (figure 2), lays eggs in groups (number varied from 2 to 85 in the laboratory). Larval development takes 3–4 weeks. The full-grown larva is a light-brown hairy caterpillar about 3 to 3.5 cm in length with orange-red mouth and creamish-yellow and brown tufts of hairs (figure 4). Pupation takes place on the plant in a thin, papery cocoon covered with larval hairs. Freshly formed pupa appears creamish-white and changes to light brown. The female pupa is bigger. Pupal period is 7 to 10 days. Caterpillars were seen defoliating mango in Lucknow from April to August in 1987.

Larval parasite *Exorista* sp. (Diptera: Tachinidae) and pupal parasite *Brachymeria lasus* Walker (Hymenoptera: Chalcididae) were recorded on this pest from Lucknow.

In view of the severe damage the pest can cause, it may be considered as a potential pest in mango orchards of Uttar Pradesh and other regions of the country.

Thanks are due to Dr Pratap Singh, Forest Entomologist, Forest Research Institute, Dehra Dun, for identification of the pest, and to Dr Z. Boucek and Dr K. M. Harris, CIE, London, for identification of the parasites.

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HARVESTING HIGH CELL DENSITIES FOR DEVELOPMENT OF *RHIZOBIUM* CELL CONCENTRATE

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A high number of viable rhizobia in inoculants is vitally important for successful use of the inoculants. Commercial inoculants, however, contain not more than 10^9 viable rhizobia per gram of wet carrier at manufacture[1]. Besides, their population declines rapidly with time, especially when inoculants are exposed to high temperature during storage and transportation, and render the inoculants ineffective. Most of the time it becomes difficult to maintain even the recommended minimum number of viable rhizobia in carrier during prolonged storage of inoculant. Stocks of cell concentrates of *Rhizobium* may overcome this problem to a great extent. They may be prepared well in advance to meet any unprecedented seasonal demand. Any type of (seed of soil) inoculants of required standard may be prepared from them just before use.

Frozen cell concentrates of *Rhizobium* strains have been used in the preparation of inoculants, particularly in North America[2]. These concentrates are easy to store and transport as they occupy approximately 300 times less space than carrier-based inoculants. They can easily be preserved either by freeze-drying or in 40% glycerol for prolonged periods[3]. In spite of many advantages, cell concentrates of *Rhizobium* are not very popular. The main reason for this is the low recovery of viable rhizobia from growth medium, which not only affects the efficiency but also the cost of production.

In this study, we assessed the potential of various growth substrates for harvesting high cell densities of *Rhizobium* from the growth medium.

Yeast extract mannitol (YEM) broth[4] was used as standard medium for comparison in all the growth experiments. However, in some experiments, mannitol, molasses, malt extract or jaggery was added (1 g) in place of 10 g per litre of mannitol. In experiments where a common source of carbon and nitrogen was used, yeast extract and malt extract were added separately (2, 6 and 10 g/l). Cultures of *Rhizobium leguminosarum* (P-3-86) and *Bradyrhizobium japonicum* (SB-16) were grown at 30 ± 2°C for 72 h on a rotary shaker (120 rpm). The cells were recovered from the medium by centrifugation at

7000 g for 20 min. The cells were resuspended in sterilized phosphate buffer (pH 7.2). The number of viable rhizobia in suspension was enumerated by plate count using a standard yeast extract mannitol agar medium containing congo red[4].

Rhizobium species are known to produce polysaccharides from sugars in growth medium[5]. This results in higher viscosity of culture medium and leads to inefficient separation of cells by centrifugation[6]. In YEM broth, though it supports good

Table 1 *Growth yields and yield recoveries of Rhizobium leguminosarum and Bradyrhizobium japonicum on different growth substrates in liquid medium*

		R. leguminosarum (P-3-86)		*B. japonicum* (SB-16)	
Nutrient	Conc. (%)	Growth yield (log cfu/ml)	Yield recovery (%)	Growth yield (log cfu/ml)	Yield recovery (%)
Mannitol	1.0	10.25	15.18 (22.87)	10.16	15.25 (22.98)
Mannitol	0.1	9.09	25.56 (30.37)	8.28	31.38 (34.08)
Molasses	0.1	8.62	28.05 (31.97)	8.56	40.69 (39.64)
Malt extract	0.1	8.10	71.00 (57.42)	7.44	72.53 (58.37)
Jaggery	0.1	8.39	32.58 (34.82)	8.59	44.84 (42.02)
		Growth yield		Yield recovery	
SE(±)		0.04		(3.78)	
CD at 5%		0.12		(11.34)	

Figures in parentheses are angular transformation values.

Table 2 *Influence of substrate concentration on growth yield and yield recoveries of Rhizobium leguminosarum and Bradyrhizobium japonicum in liquid medium*

		R. leguminosarum (P-3-86)		*B. japonicum* (SB-16)	
	Conc. (%)	Growth yield (log cfu/ml)	Yield recovery (%)	Growth yield (log cfu/ml)	Yield recovery (%)
Malt extract					
	0.2	9.03	72.18 (58.18)	7.56	72.00 (58.05)
	0.6	9.31	65.22 (53.85)	8.16	70.82 (57.29)
	1.0	9.79	62.54 (52.24)	8.70	61.48 (51.65)
Yeast extract					
	0.2	9.65	71.81 (57.81)	8.14	70.82 (57.29)
	0.6	10.20	70.85 (57.35)	9.29	68.69 (55.98)
	1.0	9.32	72.00 (58.05)	9.00	68.14 (55.61)
			Growth yield		Yield recovery
SE(±)			0.04		(3.75)
CD at 5%			0.12		(11.25)

Figures in parentheses are angular transformation values.

growth of rhizobia, the recovery of viable rhizobia is very low (less than 1%)[5]. The results of this study also show low recovery of viable rhizobia from standard YEM broth on centrifugation (table 1). Improvement was observed when mannitol was used at suboptimal level (1 g/l) in the growth medium, but the results are much better with malt extract, which gave 71–72.53% recovery of viable rhizobia in comparison to 15.18–15.25% with mannitol. Malt extract, when used as sole source of carbon, nitrogen and growth factors in the medium, was found to be better than yeast extract which has been reported to be very good for growth yield and yield recovery of *R. leguminosarum*[5]. It was, however, true only for lower concentration (2 g/l) of malt extract in liquid medium (table 2). At higher concentrations (6–10 g/l) the recovery of viable rhizobia declined, though growth yield improved. With yeast extract, however, recovery yields were good even at higher concentration.

It may be inferred from this study that a large population of viable rhizobia may be recovered from the growth medium for developing cell concentrates if malt extract is used in lower concentration as sole source of carbon, nitrogen and growth factors in the medium. This will not only reduce the cost of production (as malt extract is cheaper than yeast extract) but also improve the efficiency of production of cell concentrates because of faster recovery of cells from growth medium.

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REVERSAL OF CHLORAMPHENICOL-INHIBITED PROTEIN SYNTHESIS BY EDTA AND SULPHHYDRYL COMPOUNDS IN BHENDI SEEDLINGS (*ABELMOSCHUS ESCULENTUS* (L.) MOENCH.)

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It is well established that chloramphenicol inhibits protein synthesis on 70 S ribosomes[1]. Attempts have been made to use this inhibitor to clarify characteristics of protein synthesis in higher plants[2–4]. While chloroplast and mitochondrial ribosomes bind chloramphenicol, cycloheximide blocks protein synthesis on cytoplasmic 80 S ribosomes[5,6]. This communication presents results of experiments to test the ability of EDTA and three sulphhydryl compounds to reverse the effect of chloramphenicol in bhendi seedlings. EDTA is a metal-chelating agent and can conjugate protein[7]. Chemicals like glutathione, cysteine, ascorbic acid and thiourea have been shown to markedly reduce chromosomal aberrations caused by gamma radiation in *Tradescantia* and onion root tips[8,9]. The stability of the disulphide bond makes it an important factor in the tertiary structure of proteins[10]. Sulphhydryl groups and sulphydryl-binding reagents can participate in redox reactions by reversible formation of disulphide bonds.

Seeds of bhendi var. Pusa Savani were surface-sterilized with 5% mercuric chloride for 15 min and washed thoroughly with distilled water. They were then soaked before sowing for 24 h in 200 ppm solutions of EDTA, methionine, cysteine, glutathione or chloramphenicol or combinations of these compounds (see figure 1). Lower concentrations (50 and 100 ppm) were ineffective. Seedlings were allowed to grow in distilled water for 10 days under a light intensity of 2000 μE m^{-2} sec^{-1}. Seedling protein was extracted[11] and estimated[12] as described earlier. Increase or decrease in protein was calculated as per cent difference between control and treated seeds.

The results (figure 1) indicate that in EDTA-treated seedlings there was about 19.7% increase in protein content on day 10. Chloramphenicol treatment caused a 29.3% reduction in protein content. However, when seeds were treated with EDTA and chloramphenicol, the decrease in protein content was only 4.3% on day 10 of seedling growth. Thus

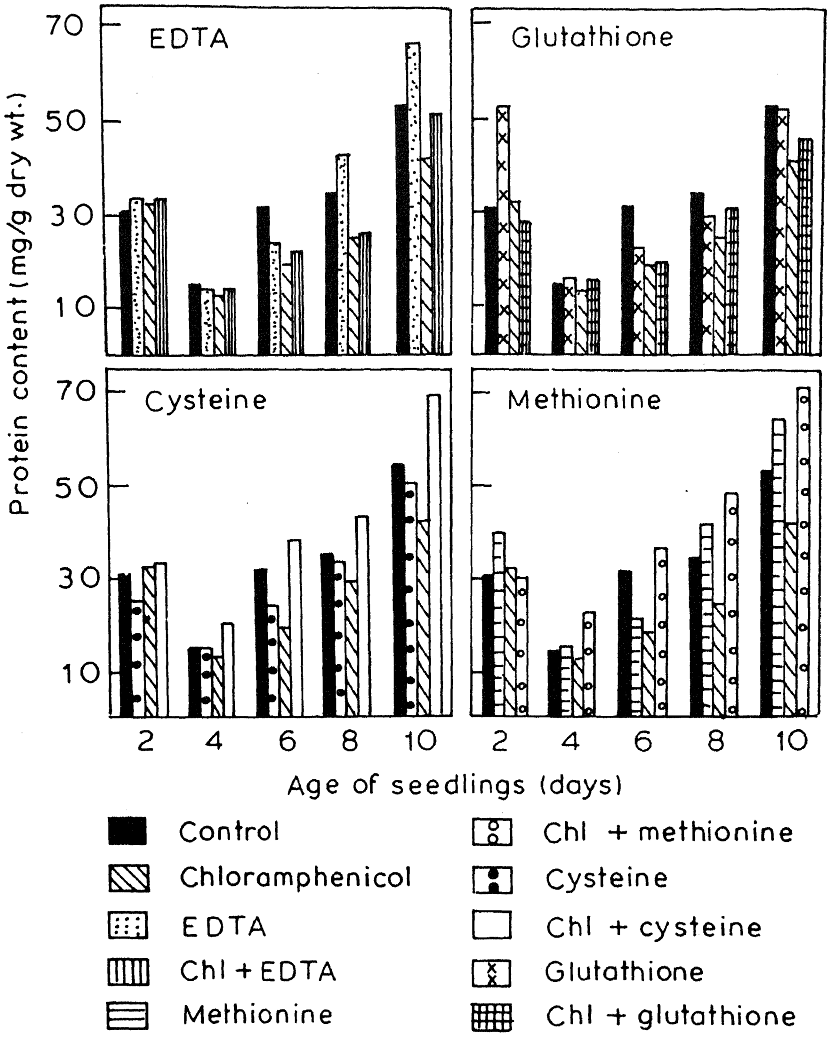

Figure 1. Protein content of bhendi seedlings from seeds treated with EDTA, glutathione, cysteine or methionine, or combinations of these reagents.

EDTA could reverse the inhibition of protein synthesis caused by chloramphenicol.

While methionine caused an increase in protein content on day 10 by about 17.1%, treatment with chloramphenicol and methionine increased protein content by 25.7%. Thus it appears that methionine is highly potent in reversing the inhibition of protein synthesis and also activating the synthesis. Earlier work[13] indicates that methionine altered the composition of the pool of free amino acids. It was also observed that methionine altered the composition of protein during sulphur deficiency. Gatehouse *et al.*[14] stated that genetic potential for increasing the ratio of the sulphur-rich protein legumin to the sulphur-poor protein vicilin, and thus improving storage protein quality by increasing sulphur-containing amino acid content, is limited. The present study might offer a strategy for the improvement of seed protein quality and quantity through a physiological approach.

Cysteine, another sulphhydryl-containing amino acid caused a decrease in protein content on day 10 by about 8.2%, but when it was used with chloramphenicol there was an increase in protein content by about 22.5%. How cysteine alone caused reduction in protein content is not clear.

According to Macnicol[15] sulphur deficiency severely affects the composition of the free amino acid pool of developing *Pisum sativum* seeds, causing a marked decrease in cysteine but no change in methionine. In the present study the response to exogenous amino acids varied with the amino acid, i.e. decrease in protein content with cysteine and increase with methionine.

Glutathione treatment caused a reduction in protein content on day 10 by about 1.3% while treatment with glutathione and chloramphenicol caused a further reduction (14.5%). However, glutathione could partially reverse the inhibition by chloramphenicol (29.3 to 14.5%). Thus, when compared to methionine and cysteine, glutathione appears to be weak in counteracting the effect of chloramphenicol. Glutathione disulphide was found to be involved in reversing inhibition of glucose-6-phosphate dehydrogenase by NADPH[16].

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PYRUVATE OXIDATION IN TOAD GASTROCNEMIUS MUSCLE AFTER ANODIC OR CATHODIC STIMULATION

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THE anatomical basis of metabolic compartmentation in the central nervous system[1-6] is attributed to neuronal elements, glial components and astrocytes. Within the neuron, regionalized compartmentation, involving perikaryon with central functions, mitochondrial elements in the axoplasm with supporting functions and terminal units with synaptic functions, has been suggested[7-9].

The metabolic systems involving oxidoreductases possess cathodal characteristics[10], while those involving protein turnover, proteases, transaminases and acetylcholinesterase activities possess anodal characteristics. These systems are demarcated in terms of cathodal or anodal charge-based metabolic compartments[11-13]. If the activity patterns of these metabolic systems are under the control of the respective polarity characteristics, exogenous supplementation of identical characteristics is expected to activate the respective metabolic events. This has been tested in the present study on toad gastrocnemius muscle.

Medium-size (30 ± 5 g) *Bufo melanostictus* were collected in and around Tirupati and were maintained in clean glass troughs containing wet sand and leaves. The bed in the troughs was changed once every day. The toads were fed cockroaches *ad libitum* and acclimatized to laboratory conditions for one week. For electrical stimulation the toads were positioned on a Perspex base with a soft rubber band and made immobile. One platinum electrode (0.2 mm thickness) was placed superficially on the skin in the region of the gastrocnemius muscle of the right leg and the other electrode (0.2 mm) was placed in the midventral region of the abdomen. The skin of the animal between the electrodes was wiped to minimize surface wetness and reduce the possibility of short-circuit effects across the skin. An electric gradient of 0.8 V/cm (DC) was applied for 5 min every day for five days. The electrode contact regions

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*For correspondence.

Oxido-reductases

were wetted with potassium chloride (0.75%). In one batch of toads, the electrode in the proximity of the muscle was made cathode and the midventral electrode was made anode. In the second batch of toads, the polarity was reversed, i.e. the muscle electrode was made anode and the midventral one was made cathode. On day 6 the toads were weighed and sacrificed quickly by decapitation. The gastrocnemius muscle of the right leg and the contralateral muscle, liver, heart and brain were excised in the cold, washed in amphibian Ringer medium[14], and allowed to stand for 10 min to recover from shock effects. The tissues were homogenized in 0.25 M sucrose and the homogenates centrifuged at 1500 *g* for 15 min. The clear supernatants were used in the studies. Pyruvate was estimated by the method of Reitman and Frankel[15], lactate by the method of Barkar and Summerson[16], and the activities of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) by the method of Nachlas *et al.*[17] The results were statistically analysed by Student's t test.

The animals in which the cathode was placed near the gastrocnemius muscle gained weight (+5.3%) while those in which the anode was placed near the gastrocnemius lost weight (−9.7%). Such weight changes after cathodic/anodic stimulation have been discussed earlier[18, 19] and shown to involve tissues such as liver, heart, brain and gastrocnemius muscle.

Pyruvate and lactate levels in the various tissues examined are given in table 1. In untreated animals, pyruvate level was lower than lactate level in all the tissues examined except heart, suggesting rapid mobilization of pyruvate into lactate. On cathodic stimulation, pyruvate level increased while lactate level decreased in all the tissues except heart, suggesting that lactate formation was diminished. This results when LDH preferentially acts in the direction lactate to pyruvate. This normally occurs when NADH content is low and NAD content is high, suggesting rapid oxidation of NADH and consequent active TCA cycle and associated oxidoreductase system.

On anodic stimulation, pyruvate content of all tissues decreased while lactate level increased (except in heart). Such a system needs a free supply of NADH and is possible when pyruvate oxidative processes are slow.

LDH is involved where anaerobic conditions favour reduction to lactate while aerobic conditions promote entry of pyruvate into the TCA cycle. Thus LDH indicates prevailing emphasis on EMP and TCA cycle systems. SDH was selected since it is the parent enzyme of the citric acid cycle. MDH was chosen in view of the catalysis involving malate where the substrate is either dehydrogenated into oxaloacetate, or undergoes decarboxylation, leading to the formation of pyruvate (catalysed by pyruvate carboxylase). Thus MDH activity reflects relative diversion of malate oxidation from the TCA cycle.

Activities of the three oxidoreductases are given in table 2. In gastrocnemius muscle from unstimulated toads, the activities were in the order SDH > LDH > MDH, suggesting that succinate oxidation takes precedence over other oxidations. The low level of oxidation of malate in terms of low MDH activity indicates a bottle-neck in the TCA cycle. The low LDH activity, compared to SDH, indicates inherent preference for TCA cycle oxidations over glycolysis under normal resting conditions.

Table 1 *Pyruvate and lactate in tissues of Bufo melanostictus after electrical stimulation of gastrocnemius muscle*

		Stimulated muscle	Contralateral muscle	Brain	Heart	Liver
Pyruvate	Control	5.64 ± 0.05	5.12 ± 0.06	3.86 ± 0.06	4.89 ± 0.075	4.36 ± 0.11
(μmol/g wt)	Cathode	7.26 ± 0.06* (+28.72)	6.20 ± 0.07* (+21.09)	4.59 ± 0.05* (+18.91)	3.81 ± 0.07* (−22.09)	5.52 ± 0.101* (+26.61)
	Anode	4.32 ± 0.06* (−23.4)	4.19 ± 0.09* (−18.16)	3.23 ± 0.06* (−16.32)	5.75 ± 0.07* (−17.59)	3.38 ± 0.102* (−22.5)
Lactate	Control	6.84 ± 0.06	6.45 ± 0.074	3.94 ± 0.056	4.28 ± 0.074	8.26 ± 0.085
(μmol/g wt)	Cathode	5.22 ± 0.064* (−23.68)	5.27 ± 0.69 (−18.29)	3.10 ± 0.051* (−21.32)	5.02 ± 0.081* (+17.29)	6.13 ± 0.091* (−25.78)
	Anode	8.05 ± 0.057* (+17.69)	7.47 ± 0.067* (+15.82)	4.56 ± 0.048* (+15.74)	3.67 ± 0.068* (−14.25)	9.94 ± 0.084* (+20.34)

Each value is mean ± SE of six observations; numbers in parentheses are per cent change over control.
*Significantly different from control ($P < 0.05$).

Table 2 *Succinate dehydrogenase, lactate dehydrogenase and malate dehydrogenase activities in tissues of Bufo melanostictus after electrical stimulation of gastrocnemius muscle*

		Stimulated muscle	Contralateral muscle	Brain	Heart	Liver
SDH	Control	1.246 ± 0.052	1.234 ± 0.035	0.986 ± 0.035	1.839 ± 0.042	1.487 ± 0.06
	Cathode	1.674 ± 0.055* (+34.34)	1.546 ± 0.057* (+25.3)	1.320 ± 0.039* (+33.87)	1.243 ± 0.044* (−32.4)	1.952 ± 0.056* (+31.27)
	Anode	0.867 ± 0.051* (−30.41)	0.956 ± 0.056* (−22.52)	0.697 ± 0.037* (−29.3)	1.198 ± 0.046* (−34.85)	1.096 ± 0.06* (−26.31)
LDH	Control	0.751 ± 0.016	0.737 ± 0.013	0.639 ± 0.02	1.216 ± 0.056	0.837 ± 0.016
	Cathode	0.978 ± 0.019* (+30.23)	0.912 ± 0.016* (+23.74)	0.854 ± 0.016* (+33.64)	0.834 ± 0.052* (−31.4)	1.137 ± 0.018* (+35.84)
	Anode	0.535 ± 0.017* (−28.76)	0.548 ± 0.015* (−25.64)	0.466 ± 0.017* (−27.07)	0.854 ± 0.055* (−29.76)	0.617 ± 0.021* (−26.28)
MDH	Control	0.429 ± 0.022	0.416 ± 0.014	0.314 ± 0.026	0.516 ± 0.019	0.542 ± 0.007
	Cathode	0.547 ± 0.026* (+27.5)	0.507 ± 0.038* (+21.87)	0.397 ± 0.025* (+26.43)	0.390 ± 0.018* (−24.40)	0.735 ± 0.01* (+35.61)
	Anode	0.320 ± 0.025* (−25.4)	0.317 ± 0.013* (−23.79)	0.238 ± 0.024* (−24.2)	0.415 ± 0.018* (−19.57)	0.365 ± 0.011* (−32.65)

Activity expressed as μmol of formazan/mg protein/h.
Each value is mean ± SE of six observations; numbers in parentheses are per cent change over control.
*Significantly different from control ($P < 0.05$).

On cathodic stimulation, all three activities increased in all tissues except heart, but the increase in MDH activity was smaller. This indicates that pyruvate oxidation through TCA cycle and LDH system favour NAD-dependent pyruvate formation.

Anodic stimulation resulted in decrease of activity of all three enzymes in all the tissues. The decrease was between 20 and 30%, and indicates pyruvate oxidation through TCA cycle and increased NADH-dependent lactate formation by LDH. The response of cardiac tissue, which showed decrease in LDH, SDH and MDH activities after both cathodic and anodic stimulation, was unique, probably indicating some fatigue resistance inherent in the heart.

In summary, cathodic stimulation elevated oxidoreductase activities, pyruvate oxidation and consequent energy production, with possible contribution to the endergonic reaction systems, while anodic stimulation caused decreased pyruvate oxidation and decreased oxidoreductase activity, and consequent diminished cellular energy production.

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MYOCARDIAL GLYCOGEN CONTENT OF SOME REPRESENTATIVE VERTEBRATES

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THE polysaccharide glycogen is the immediately available fuel reserve for energy metabolism along the glycolytic pathway. In muscle, including cardiac muscle, it has considerable physiological significance, especially during hypoxia, and is observed to vary in level under diverse physiological conditions[1-5]. Further, chamberwise variations in heart glycogen content are also reported in some vertebrates[2,6]. Nevertheless, data on glycogen content of myocardium of vertebrates adapted to diverse ecological conditions and activity levels are scant. Accordingly, it was thought that a study on the glycogen content of the different chambers of the heart of some representative vertebrates would yield valuable information regarding the adaptive physiology of vertebrate myocardium with reference to glycogen.

Nine representative vertebrates, adapted to diverse ecological conditions and activity levels, were chosen for the present study. Myocardial samples were excised from healthy adult animals of both sexes and comparable body size. Glycogen was estimated by the method of Seifter *et al.*[7] using anthrone reagent. The data are given in table 1.

In general, lower vertebrates show higher glycogen content in the myocardium than birds and mammals. Further, variation between the chambers is much more in lower vertebrates than in birds and mammals. This probably indicates that the carbohydrate fuel reserve, glycogen, has a greater significance in the physiological adaptation of the myocardium of lower vertebrates than that of birds and mammals.

Glycogen content of myocardium of *Cybium* is not so high in comparison with that of some other lower vertebrates. In fact, relatively higher glycogen values are seen in the myocardium of *Rana*, with significant regional variation, especially between the two ventricular halves, the right half showing a higher glycogen content than the left. This may be due to greater muscular effort of the right ventricular half in pumping venous blood to the pulmonary and cutaneous respiratory surfaces. Functional differentiation of the right and left halves

Table 1 *Glycogen content of myocardium of the different chambers of heart in nine vertebrates*

	Glycogen (μg/100 mg wet tissue) Atrium		Ventricle	
	Right	Left	Right	Left
Cybium guttatum	109.61 (6.52)		84.24 (5.34)	
Rana tigrina	354.16 (24.65)	329.46 (25.91)	758.58 (69.99)	473.14 (51.14)
Calotes versicolor	143.45 (13.63)	147.42 (14.41)	83.72 (15.51)	58.39 (9.02)
Geomyda trijuga	112.97 (7.21)	52.83 (5.11)	82.42 (6.24)	61.67 (4.51)
Lissemys punctata	2145.61 (121.07)	1605.23 (50.78)	1561.56 (70.88)	1904.14 (143.53)
Gallus domesticus	58.56 (3.71)	26.74 (2.63)	35.72 (2.59)	24.81 (1.21)
Columba livia	36.66 (2.28)	29.65 (2.82)	23.55 (3.57)	18.16 (1.21)
Carpa sp.	98.81 (5.58)	127.64 (11.58)	94.81 (7.02)	136.73 (9.57)
Pteropus giganteus	40.95 (5.21)	31.94 (2.97)	26.42 (1.73)	15.78 (1.21)

Values are the mean of ten assays with standard error in parentheses.
Variance analysis was carried out and the F ratio was calculated to test the significance of variations. Significant variation is observed between animals ($P < 0.01$) and between chambers ($P < 0.01$).

Anaerobic glycolysis; vertebrate myocardium of the ventricle of amphibians has been reported by Johansen and Hansen[8].

Among reptiles, *Calotes* and *Geomyda* do not show significant variations in glycogen content of ventricular myocardium. Unlike *Lissemys*, which is a diving form, *Calotes* and *Geomyda* do not confront prolonged anoxia and may not be in need of large stores of glycogen. However, significant chamberwise variations are observed in the myocardium of *Lissemys*. In the present study, the highest glycogen values were obtained for the myocardium of *Lissemys*, followed by that of *Rana*, which are diving forms. High glycogen has also been reported for the myocardium of another diving turtle, *Chrysemys picta belli*[2]. Clark and Miller[3] attributed the anaerobic survival of the freshwater turtle *Pseudomys scripta elegans* to low metabolic requirement and capacity for sustained anaerobic glycolysis. Reeves[9] has shown that turtle hearts are capable of tolerating sustained anaerobiosis for prolonged periods, with glycogen the preferred substrate for myocardial energy supply.

In general, glycogen content of myocardium of birds and mammals are lower. No significant variations in glycogen level have been observed between *Gallus* and *Columba*. In fact, the myocardium of both species shows an oxidative type of metabolism, as evidenced by higher SDH activity with lipid as fuel[10]. Interestingly, the flying form *Pteropus* showed a much lower glycogen content than the less active and domesticated form *Capra*. High phosphorylase activity has been shown in the myocardium of *Pteropus*[11]. Probably, being a flying mammal, *Pteropus* needs greater glycogen turnover than *Capra*. Further, diurnal dormancy and subsequent bradycardia are generally observed in bats[12]. *Pteropus* also exhibits diurnal dormancy, and probably its myocardium depends on glycogen metabolism for energy as a result of physiological adaptation to daily torpor.

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MUTAGENIC POTENTIAL OF HUMAN KALA-AZAR HAEMOFLAGELLATE IN MOUSE

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WHILE the mutagenic potential of numerous chemical and physical agents in mice and other mammalian models is an established fact, the idea of microbes like viruses, mycoplasma, bacteria and fungi imperfecti acting as 'living mutagens' has also been proposed and examined[1-7]. Protozoans have not been similarly examined[8]. This communication extends the study to a protozoan parasite.

Leishmania donovani, the human kala-azar parasite, was cultured in diphasic Tobi's solid slant medium[9] and isolated in phosphate buffer, pH 7.2. One ml of a suspension containing approximately 5×10^7 parasites was injected intraperitoneally into Swiss albino mice (body weight about 20 g). Control mice, from the same stock, were given 1 ml of phosphate buffer. The parameters used to test the mutagenic potential of *L. donovani* in mouse were bone marrow chromosome aberrations, micronucleus test (MNT), frequency of sperm with abnormal head morphology and sperm depletion effect.

Bone marrow chromosome preparations were made 6 h and 24 h after injection following the standard colchicine–sodium citrate–acetic alcohol–flame drying–Giemsa staining schedule[10]. Two hundred metaphases from control and parasite-injected mice were examined for chromosome aberrations.

For MNT, bone marrow was collected 24 h after the injection in 1% sodium citrate solution. Unfixed cell suspension from bone marrow was smeared on a clean grease-free slide, stained first in May–Grunwald stock solution for 3 min, immediately followed by staining again in distilled water-diluted May–Grunwald stain (1:1) for 2 min, and then finally stained further with diluted Giemsa stain (1:6, pH 6.8) for 10 min[10]. The stained slide was rinsed with water and air-dried. The frequency of micronucleated erythrocytes in 2000 poly- (bluish texture) and normochromatic (reddish texture) forms per mouse was assessed.

Sperm samples were prepared by squeezing out sperm from the tubules of epididymis of each side into 2 ml of distilled water. The sperm suspension was filtered through silk cloth to remove debris and the filtrate was mixed with more distilled water to make it up to 8 ml (ref. 10). The sperm suspension was smeared on a clean slide, air-dried, stained in distilled water-diluted Giemsa stain (1:20, pH 6.8), and finally air-dried[10]. The frequency of sperm with abnormal head morphology in 2000 sperm per male mouse was assessed. The same sperm suspension samples were used for sperm depletion test. Drops of the suspension were placed in five grooves of a Neubauer slide and the number of sperm in each groove was counted. The mean number was arrived at from five replicas of epididymis of each side.

The bone marrow cells of control mice had a very limited range of types of aberrations. True break types were practically absent. There were quite a number of cases of precocious centromeric separation and some grossly affected metaphases. The total aberration frequency from 400 metaphases (6 h and 24 h) was 8.2%, and only 1.5% (comprising 1 acentric fragment and 5 polyploids) had some genetic significance (table 1). On the other hand, in parasite-injected mice, the bone marrow chromosome aberrations were in the form of subchromatid (figure 1) and chromatid breaks (figure 2), acentric fragment (figure 2), translocations like ring (figure 1), centric fusion (figure 3), physiological gap and constriction, precocious centromeric separations (figure 2), etc. among individual chromosome anomalies, while the grossly affected metaphases included polyploidy (figure 4), aneuploidy, pycnosis, stickiness, etc. (table 1). Among individual chromosome anomalies, precocious centromeric separation was most common (table 1), and occurred overwhelmingly in chromosomes of group V, as found elsewhere[11]. Injection of *L. donovani* induced strikingly high increase in frequency of chromosome aberrations (table 1).

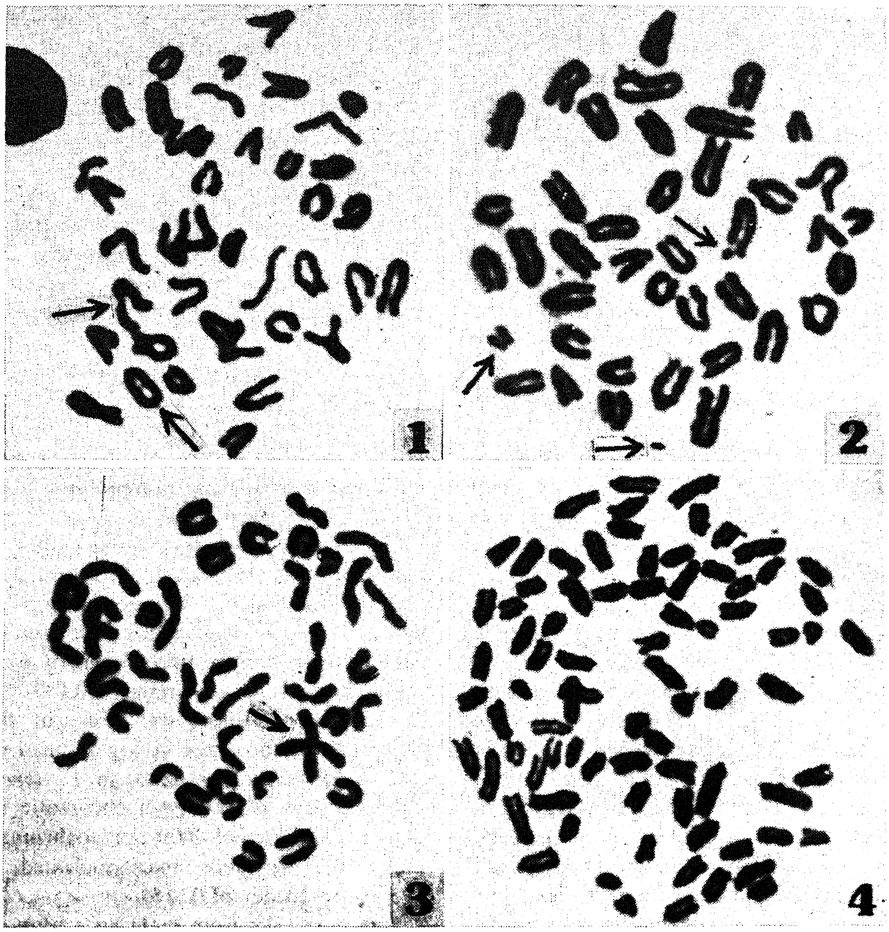
The micronucleus test showed one micronucleus per micronucleated bluish polychromatic (figure 5) and reddish normochromatic (figures 7, 8) erythrocytes; rarely two micronuclei were seen (figure 6). In samples from control mice, 4 out of 4000 polychromatic erythrocytes examined (0.1%) were micronucleated while there was none in 4000 normochromatic erythrocytes, giving a mean frequency of 0.05%. On the other hand, in *L. donovani*-treated mice, 32 out of 4000 polychromatic erythrocytes (0.8%) and 9 out of 4000 normochromatic erythrocytes (0.22%) were micronucleated, giving an average frequency of 0.51%.

Sperm samples from male mice were examined at 6 and 24 h after injection. Thirty-two out of 2000 sperm (1.6%) were abnormal in head morphology (figures 10–12; compare with figure 9) in samples from control male mice and 248 out of 2000 (12.4%) in samples from parasite-injected mice 6 h after injection. At 24 h the frequency of abnormal sperm

Table 1 *Chromosome aberrations in bone marrow cells of normal and L. donovani-injected Swiss albino mice*

Time after injection	Group	No. of metaphase counted	No. of metaphase affected	Individual types						Gross types		Total aberrations	
				Scb	Cb	Af	Tr	C & G	Pcs	Number	Other	(No.)	(%)
6 h	Control	200	17	—	—	—	—	—	14	2	1	17	8.5
	Treated	200	50	3	8	2	—	3	30	4	5	55	27.5
24 h	Control	200	15	—	—	1	—	—	9	3	3	16	8.0
	Treated	200	44	2	6	4	4	8	17	7	10	58	29.0
Total	Control	400	32	—	—	1	—	—	23	5	4	33	8.2
	Treated	400	94	5	14	6	4	11	47	11	15	113	28.2

Scb, Subchromatid; Cb, chromatid break; Af, acentric fragment; Tr, translocations and ring; C & G, physiological gap and constriction; Pcs, precocious centromeric separation.

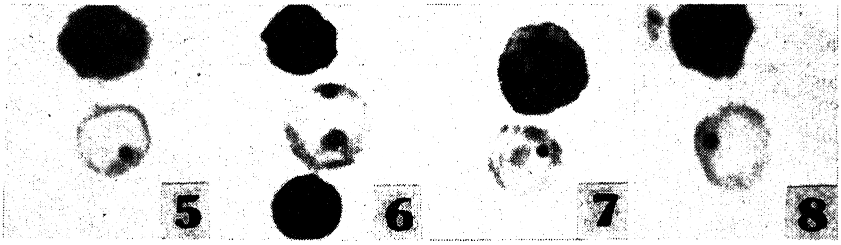


Figures 1–4. Bone marrow chromosome aberrations induced by *L. donovani* in Swiss albino mice: **1,** a ring and a subchromatid break; **2,** a chromatid break, an acentric fragment and precocious centromeric dissociation in a group V chromosome; **3,** a centric fusion; **4,** a polyploid metaphase.

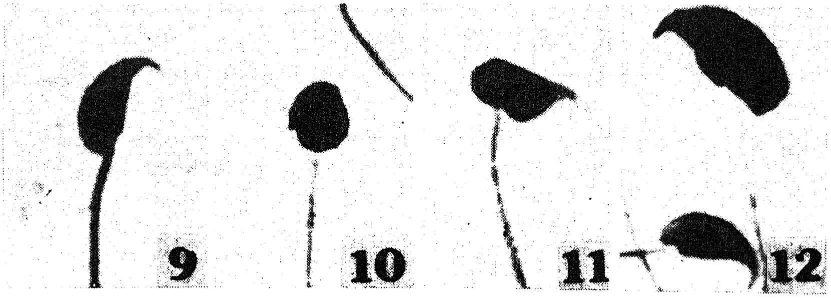
was 1.5% (30/2000) in control and 18.9% (378/2000) in parasite-injected mice.

The mean number of sperm was 1.2 and 1.1 at 6 h, and 1.2 and 1.6 at 24 h in right and left epididymes respectively of control males, but 0.84 and 0.84 at 6 h, and 0.96 and 0.84 at 24 h in the right and left epididymes of *L. donovani*-injected mice. This indicates some sperm depletion in the parasite-injected mice.

It has been recommended[12] that to consider an



Figures 5–8. Micronucleated erythrocytes from *L. donovani*-injected mice.



Figures 9–12. Normal sperm (9) and sperm with abnormal head (**10–12**) from *L. donovani*-injected male mice.

agent as mutagenic, positive result in more than one test is desired. In the present study results were positive with regard to bone marrow chromosome aberration frequency, MNT and frequency of abnormal sperm. Although injection of *L. donovani* did not bring about a marked sperm depletion effect, it is reasonable to suggest a mutagenic potential for *L. donovani* in mouse. Therefore the present study extends the concept of 'living mutagens', claimed earlier for viruses, bacteria and fungi, to a protozoan parasite. However, the precise mechanism by which *L. donovani* may act as a mutagenic agent is unknown. It has been shown[6, 8] that toxins and vaccines can act as clastogenic agents. However, this must be carefully verified.

It is debated whether sperm head morphology is under genetic control but the idea is favoured by Wyrobek[13]. The effect was found in F_1 and F_2 generations of mice treated with chemicals, bacteria and fungi[6-8]. Use of sperm head morphology as a criterion in mutagenicity testing has been adopted by many workers. Chromosome aberrations have remained the most authentic indicator of mutagenicity[14]. MNT has also become a standard technique and is used widely.

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BOOK REVIEW

Annual Review of Genetics, vol. 22, 1988, pp. 704, (eds) Allan Campbell, Bruce S. Baker and Ira Herskowitz, (Published by Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California 94306, USA), Price: USA $ 34, Elsewhere $ 38.

The present volume of the *Annual Review of Genetics*, in its traditional format, contains 22 chapters, all of them written by leading workers in their areas. Four of the articles are in the area of plant genetics, while nearly 10 of them deal with various aspects of mammalian genetics. The remaining articles are devoted to various other systems like phages, viruses and microorganisms, and specific molecular aspects.

The first review is titled 'Basic processes underlying *Agrobacterium*-mediated DNA transfer in plant cells' (P. Zambryski). While *Agrobacterium* can be routinely used as a vector to transfer DNA to plant cells, the 'biology' of the system presents a number of interesting problems. The aspects presented here pertain to the stages between plant cell recognition and transformation (which represent the T-DNA transfer process), comprising at least seven steps. The tomato is a favourite model system for both classical and molecular genetics (C. M. Rick and J. I. Yoder). It is a simple diploid; its twelve chromosomes have been well mapped. Several conventional and molecular markers are available and hereditary variations are easily detectable. Besides, excellent stock collections are available (germ plasm), and there are more such advantages. The molecular mechanisms regulating fruit ripening and developmental processes such as genesis of leaf shape, flower development, seed germination and stomatal behaviour are topics of current and future research using this system. In the article on maize developmental genetics (W. F. Sheridan), the central problem in the developmental biology of plants, viz. the role of genes in morphogenesis, is reviewed. Which are the genes and what mechanisms do they employ in directing and regulating morphogenesis from fertilization to embryo maturation, and from embryo germination to gamete formation are the major themes for discussion. The review entitled 'Foreign genes in plants: transfer, structure, expression and applications' (Weising, J. Schell and G. Kahl) examines the new insights into natural plant engineering and the impact of plant genetic engineering for basic gene research as well as crop improvement. An entire list of higher plant species transformed by gene transfer techniques has been provided here for ready reference.

There are two articles relating to sex determination and differential imprinting in mammalian systems. The article 'Mechanisms of X-chromosome regulation' (S. G. Grant and V. M. Chapman) discusses the molecular mechanisms governing the transition from single X expression in primordial germ cells and oogonia to an oocyte with two active X-chromosomes, and from embryonic cells with 2 active Xs to somatic cells with a single X. There are still many unresolved questions in this problem. It has become clear recently that mammalian development requires the functional presence of both maternal and paternal genomes. The relative importance of each gamete for subsequent development and the nature of interaction between gametes remain basic questions in developmental biology. Classical genetic analysis of meiotic nondisjunction, or manipulative techniques of nuclear transfer have revealed that some genes are differentially expressed, depending on whether they are contributed by the maternal or the paternal gamete. The possible mechanisms of differential expression are discussed by D. Solter in the article 'Differential imprinting and expression of maternal and paternal genomes'.

Mammalian genome organisation: an evolutionary view' (S. J. O'Brien, H. N. Seuanez and J. E. Womack) discusses the emergence of a cohesive picture of chromosome evolution in mammals. The application of molecular techniques to genetic analysis has provided composite genetic maps of several mammalian species including man. Today, about 3500 specifically localized markers on the human chromosomes are known, of which 1500 are functional genes. A 5–10-centimorgan human recombination map has been developed. A multidisciplinary 'genome' project designed to determine the entire DNA sequence of the human genome (approx. 3.2×10^9 base pairs) is on, and the latest timetable is to complete a 1–3-centimorgan linkage map in 3–5 years and a complete DNA sequence in 15 years. Technologies similar to those employed in human genetics have also been used to construct gene maps in several other mammalian species (primates, rodents, carnivores and a few others like cow, horse, pig and sheep). The discovery of a powerful set of new tools for gene mapping by linkage based on the fact that informative genetic markers could be constructed with a combination of restriction enzymes and cloned DNA probes (RFLP technique) has revolutionized human genetics. The review entitled 'Sets of linked genetic markers for human chromosomes' (R. White and J. Lalouel) discusses DNA markers for the human genetic map, and the medical and forensic applications of maps of genetic markers. The latter aspect deals with both low-density and high-density mapping of genes that cause diseases; characterization of tumour chromosomes; and identification of perpetrators of crimes and biological parents, an important component of forensics. A review on tumourigenesis is provided by D. Hanahan. The development of a malignant tumour is a multistep process composed of a series of separable changes rather than a single deleterious change. The discovery of dominant transforming genes capable of inducing tumours following infection of animals or morphological transformation of animal cells *in vitro*, as well as the establishment of methods to transfer genetic information stably into the germ lines of laboratory mouse have provided powerful tools to investigate the consequence of expression of these genes on specific cells in the context of the whole organism. It is evident that inappropriate expression of a variety of oncogenes can result in the inevitable development of tumours and that there is an apparent specificity in oncoprotein action. One clear difference between tumours in humans and those in transgenic mice is that the former are often invasive and highly metastatic whereas metastasis is rare in tumours seen in transgenic mice.

There are four reviews covering the area of genetic disorders. 'Genetics of Duchenne muscular dystrophy' (R. G. Worton and M. W. Thompson) discusses the current understanding of this X-linked lethal disease that for many years was the most perplexing and frustrating disorder in clinical genetics. Prospects for understanding the disease changed dramatically after the gene responsible for Duchenne muscular dystrophy was mapped to a specific region of the X-chromosome and genomic sequences and cDNA clones were isolated. This was followed by the identification of dystrophin, the gene product of the normal allele. 'HLA disease associations: models for insulin-dependent diabetes mellitus and the study of complex human genetic disorders' (G. Thomson) discusses HLA and disease association as a model for the study of genetic disorders. 'Mendelian hyperphenylalaninemia' (C. R. Scriver, S. Kaufman and S. L. C. Woo) deals with the genetic and biochemical aspects of the syndrome in man. This disease can be prevented either by provision of a surrogate enzyme or by somatic cell gene therapy in phenylketonurea and by more effective cofactor therapy in certain types of phenylalaninemia. Families at risk will probably avoid the problem altogether by means of prenatal diagnosis and genetic counselling. According to these authors, genetics, medicine and society meet in hyperphenylalaninemia. In his review on aneuploidy, C. J. Epstein has covered the principles, precedents and experimental evidence guiding us in our search for the mechanisms. Aneuploidy is a frequent cause of mental retardation, congenital malformation and pregnancy wastage in humans. It also plays an important role in the pathogenesis of malignancy. Aneuploid conditions are the product of the simultaneous abnormality of multiple genes (two or many) and the phenotype is a consequence of the imbalance of several genes. They are manifestations of abnormalities of gene dosage; however, a mechanistic link has not yet been forged between any phenotypic feature of an aneuploid state and an imbalance of a specific locus.

Molecular and general aspects of recombination are presented in three reviews. The mechanism of conservative site-specific recombination (N. L. Craig) occurring within a short region of sequence identity shared by participating DNA segments by reciprocal exchange of strands by precise breakage and joining is contrasted with transposition, which occurs with accompaniment of DNA synthesis. Information on how the widely separated DNA sites communicate with one another and the chemistry of strand exchange is increasing. Recombination in prokaryotes, yeast and bacteriophage are covered in the other two reviews by T. D. Peters and C. W. Hill, and D. S. Thaler and F. W. Stahl, respectively.

One of the most important topics in genetics is the regulation of gene expression. In this area there are three articles in the current volume. 'Control of antigen gene expression in African trypanosomiasis' (E. Pays and M. Steinert) describes the strategy adopted by the parasitic microorganism to avoid the immune response of its host, of switching the expression of its surface antigens. A combination of two mechanisms intrinsic to the telomeric structures provides the parasite with an enormous variation potential with the available repertoire of antigen genes. The review 'Spliceosomal snRNAs' (C. Guthrie and B. Patterson) describes the role of the small nuclear RNAs in splicing reactions, with the help of structural models and principles of alignment. Biological regulation by antisense RNA (review by R. W. Simons and N. Kleckner) has also attained importance in recent years because of the role of antisense RNA in control of plasmid copy number as well as in regulation of expression of specific genes. Again, this article is presented with structural models to make understanding easier.

The other review articles in this volume are 'The genetics of bovine papilloma virus type 1' (P. F. Lambert, C. C. Baker and P. M. Howley), 'Phylogenies from molecular sequences: inference and reliability' (J. Felsenstein) and 'Heat shock proteins' (S. Lindquist and E. A. Craig). Each of these reviews gives adequate coverage of the respective topics.

It is clear that the present volume covers a wide variety of topics. The fact that is most evident is that the concerted research efforts on the mammalian genome in the past few years have started yielding newer insights into such complex problems as sex determination, genetic imprinting, the molecular basis of tumorigenesis and genetic disorders. The book is an extremely useful one in any reference collection.

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RESEARCH GRANTS FROM CENTRAL SILK BOARD

The Central Silk Board is implementing the National Sericulture Project at a total cost of Rs. 555.3 crores spread over a period of five years from 1989 with financial assistance from the World Bank and the Swiss Development Co-operation. The collaborators of the project will the Central Silk Board are the five traditional silk-producing States of Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal and Jammu and Kashmir.

The main thrust of the National Sericulture Project is directed towards providing R&D support to the growth of Sericulture and Silk Industry. In order to achieve this objective, the National Sericulture Project provides funds to Universities to support research programmes in Sericulture and Silk Technology. This scheme is centrally administered by the Central Silk Board.

Universities interested in availing this opportunity may obtain further details from:

The Member Secretary,
Central Silk Board,
II Floor, United Mansions,
No. 39, M.G. Road,
Bangalore 560 001

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HOMI BHABHA FELLOWSHIPS

Homi Bhabha was born in Bombay eighty years ago on the 30th October 1909.

On the 24th January 1967, to mark the first anniversary of his tragic death in the aircrash of the Air-India plane on Mont Blanc, Mr J. R. D. Tata as Chairman of the Tata Institute of Fundamental Research and also of the Dorabji Tata Trust, announced the establishment of a new Foundation bearing Dr Homi Bhabha's name and dedicated to a purpose close to his heart.

In India, as in other countries, there is a great and continuing need for men and women of exceptional qualities of mind and character to provide enlightened leadership. The Homi Bhabha Fellowships Council accordingly seeks out and assists outstanding young persons at a stage in their careers when the scope normally available for the full development of their talents may be restricted by their relatively junior position in their profession or in the organization to which they belong. It is the object of the Council to provide such persons with the means and opportunities for the full development of their abilities at an age when they are at the height of their energy and mental powers, thus enabling them to make creative and original contributions and provide the leadership required in various spheres of national life.

The Foundation was named after Homi Bhabha not only to honour his memory but because his genius and vision served to inspire and encourage young people to strive for excellence and to aim at significant achievements in the service of India. The Homi Bhabha Fellowships Programme is thus intended to carry forward into various fields of endeavour the inspiration which Dr Bhabha had so effectively provided in the great tasks and projects which he formulated and brought to fruition. In the words used by Sir John Cockcroft, Fellow of the Royal Society and Nobel Laureate, in the course of his Homi Bhabha Lecture to the Royal Institution in London in January 1967:

> Human progress has always depended on the
> achievements of a few individuals of outstanding
> ability and creativeness.

In the twentytwo years since its establishment, more than 75 Homi Bhabha Fellowships have been awarded to distinguished young men and women in all fields of human endeavour, including scientists, engineers, agronomists, architects, educationists, historians, social scientists, writers and artistes. Many of them have made their mark in public life.

A Fellowship is awarded normally for a period of one to two years and its value ranges between Rs. 4,000 and Rs. 6,000 per month, with funds, where necessary, provided for equipment and other requirements and travel within the country or abroad. The Government of India has granted full exemption from income tax to the recipients of Homi Bhabha Fellowships.

The Chairman of the Homi Bhabha Fellowships Council, Mr J. R. D. Tata, its Vice-Chairman, Mr J. J. Bhabha, and its Honorary Executive Director, Prof. B. M. Udgaonkar, are keen particularly at this juncture that more than the usual number of Fellowships be awarded in the next twelve months to young men and women of exceptional talent and dedication.

Applications for Fellowships should be directed to: Hon. Executive Director, Homi Bhabha Fellowships Council, C/o National Centre for the Performing Arts, Dorabji Tata Road, Nariman Point, Bombay 400 021.

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The Science and Engineering Research Council (SERC) of the Department of Science and Technology (DST) organized a series of seminars to review and update thrust areas of research in various fields. Seminars were held in: engineering sciences (Jamshedpur, 18–20 November 1988); chemical sciences (Baroda, 24–25 November 1988); life sciences (Lucknow, 6–8 December 1988); physical sciences (Santiniketan, 23–25 February 1989); earth sciences (Dehra Dun, 4–6 August 1989); atmospheric sciences (Pune, 16–18 August 1989).

In the April 20 issue we published the recommendations of the seminar on chemical sciences. In this issue we publish the recommendations of the seminar on life sciences. The introduction to the report gives a background to the approach of SERC, with special reference to life sciences. The report is divided into seven parts representing seven separate reports prepared by the Programme Advisory Committees. This is followed by general recommendations of the seminar. We hope that the issues raised in the report and the recommendations made by the scientific community would open up a national debate on this approach to supporting scientific research in the country. Scientists are welcome to write to Current Science or Adviser (Science and Technology Promotion Division), Department of Science and Technology, New Delhi, regarding suggestions on implementation of the programmes.

—Editor

CHALLENGING AREAS IN LIFE SCIENCES

INTRODUCTION

An important component of the allocation of business of the Department of Science and Technology is 'Promotion of new areas of science and technology'. To fulfil this obligation and to focus national efforts in some specific emerging areas, DST organized a series of national seminars at Baroda during 1980–83. These seminars were in the form of well-prepared debates and provided guidelines for supporting R&D activities. These seminars, now known as the 'Baroda Seminars' identified 'thrust areas' in all branches of science and engineering for support on a preferential basis. The broad guidelines for identification of thrust area programmes were that these should

- —lead to growth of indigenous research capabilities
- —be challenging to scientists
- —be relevant to overall socio-economic development.

To encourage activity in these chosen areas it was planned to

- —initiate major coordinated research programmes
- —set up core groups/units around scientists
- —organize training programmes and summer and winter schools with the help of outstanding scientists
- —establish national support facilities in selected areas of high technology.

It may be pointed out that this exercise coincided with unparalleled developments in biological sciences. Molecular biology, genetics, biotechnology and immunology were emerging as new disciplines, with tremendous possibilities of enhancing our knowledge of biological phenomena and immediate application in agriculture, animal and medical sciences.

To initiate, implement and monitor these programmes, DST constituted a Programme Advisory Committee (PAC) in each of the major areas. In the field of life sciences, PACs were constituted in

- —molecular biology
- —molecular biophysics and biochemistry
- —plant sciences
- —animal behaviour, ecology and evolution
- —medical biology
- —immunology
- —neurobiology and mechanisms of behaviour.

DST has an apex advisory body, viz. the Science and Engineering Research Council (SERC), consisting of expert scientists and engineers, to evolve policy guidelines, determine priorities and coordinate the activities of all the PACs.

As a result of these efforts initiated in the early eighties, substantial support has been provided to these selected programmes. During the last year or two, through a series of group monitoring workshops, involving PAC members, experts and principal and young investigators of projects, an evaluation of completed and ongoing programmes was carried out.

MOLECULAR BIOLOGY

1. PREAMBLE

BIOCHEMISTRY in the international scene evolved as an interdisciplinary area in the beginning of the 20th century. India started with a strong base in nutrition biochemistry and enzymology. Molecular biology emerged as a branch of biochemistry and soon strongly evolved to have a distinct identity. This happened in the fifties on the international scene and with a gap of a decade in our country. The seventies have seen a strong emergence of genetic engineering in the West. Although, the term signifies a particular technology or methodology, the area has broadened enormously to signify a particular approach to understand the molecular basis of life processes. In other words, molecular biology as such is becoming old-fashioned unless a recombinant DNA approach and the use of the modern nucleic acid methodologies are taken to study the problems. The advent of recombinant DNA research and the associated technologies have revolutionalized the approach used in present day molecular biology and biochemistry. The entire research is an operation in microscales and gadgets have also been developed to handle micro, pico and nano levels of macromolecules. Knowledge about sequence of amino acids in proteins is more easily obtained from the corresponding DNA sequence of appropriate cloned DNA. Active sites of enzymes are more easily and specifically altered by site-specific mutation of the corresponding DNA sequences. Thus, the impact of this technology revolution permeates all disciplines of biology. Thus, our effort at redefining thrust areas has to recognize the revolution that has taken place in terms of a totally new approach to understand and define the molecular basis of life processes.

While, this is the picture that emerges when one is discussing basic biology, an unprecedented situation has also arisen in view of the possible applications, which modern biology holds for the betterment of the quality of life. Thus, new possibilities for treatment and cure of infectious diseases and genetic disorders, control of population explosion, improvement of crop plants and other components of the plant kingdom, a new approach to industry and environment management have arisen. While, the technology content in the new approach cannot be very different between countries, the choice of systems to work with can be totally indigenous, dictated by the needs of our society. Thus, we have an opportunity to combine scientific curiosity and the passion to understand basic biological phenomena with the mundane needs of our society, where a scientist can also legitimately feel proud that he/she has also contributed towards the alleviation of societal suffering.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

The Baroda seminar resulted in the formation of a Programme Advisory Committee for molecular biology and cellular biology/genetic engineering. At this stage it is rather difficult to sift the projects classified in molecular biology from those belonging to cell biology. Nevertheless, a total of 35 projects were funded during the period and the total amount constituted large per cent of the funds given to life science research.

An indication of the molecular biology carried out in the earlier part of the period can be obtained from an extract of the SERC review containing research highlights for the period 1980–87. It highlights the following aspects:

2.1 Binary complex formation between 16S and 23S RNA as a basis for ribosome assembly
2.2 DNA repair mechanisms in *Vibrio cholerae*
2.3 Methylation/demethylation processes in gene activation in *Drosophila*
2.4 Mitogenic factor in rat testes and its regulation by vitamin A levels.

These are only examples to emphasize the conventional molecular biology that formed the basis of research output in early 1980s, which also resulted in high quality research in a few pockets.

As a follow-up of the Baroda seminar, institutions were invited to submit proposals in the area of recombinant DNA research and ultimately five institutions established Genetic Engineering Units. These were at (i) Bose Institute, Calcutta, (ii) Jawaharlal Nehru University, Delhi, (iii) Indian Institute of Science, Bangalore, (iv) Madurai Kamaraj University, Madurai and (v) Banaras Hindu University, Varanasi. In addition, several specific proposals in molecular biology, where the use of recombinant DNA research has formed the thrust were funded in many institutions. The outcome of this effort is very impressive. From a situation in 1980, where hardly any group could have boasted of a set-up to use recombinant DNA technology, a recent symposium entitled 'Genetic Engineering Research in India' held at Bangalore (July 26–28, 1988) could elicit the participation of about 50 research groups from all over the country. DST funding is largely responsible for this build-up, although recently the Department of Biotechnology has taken over some of the centres and is also funding specific proposals.

The areas, where some competence has been built are:

- molecular biology of disease-producing organisms;
- Basic understanding of eukaryotic gene structure and expression in animal and plant systems;
- molecular biology of organisms of importance in agriculture;
- molecular basis of special phenomena such as DNA repair and recombination, nitrogen fixation, osmoregulation, etc.;
- development of DNA-based probes as diagnostics.

To site specific examples:

A. At the Bose Institute, Calcutta, a strong group has evolved looking at the plant tubulin genes and ribosomal RNA genes in fish and other systems.

B. At the Indian Institute of Chemical Biology, Calcutta, molecular biology of *Leishmania* and *Vibrio cholerae* are being looked at in great detail.

C. At the Indian Institute of Science, Bangalore, structure and expression of genes from liver (genes concerned with biotransformation of chemicals), silk moth and rice have been studied in detail. The complex steps involved in meiotic recombination in eukaryotic systems have been unravelled.

D. At the Jawaharlal Nehru University, Delhi, nitrogen-fixing genes in *Azotobacter* and molecular biology of *Candida* yeast have been investigated.

E. At the Centre for Cellular and Molecular Biology, Hyderabad, significant progress has been made in the understanding of bacterial genes involved in osmoregulation.

F. At the Bhabha Atomic Research Centre, Bombay, the phenomenon of male sterility in plants has been investigated and exploited.

G. The Madurai Kamaraj University, Madurai, and the University of Poona, Pune, have evolved into good centres for teaching M.Sc. programme in molecular biology.

H. At the National Institute of Immunology, Delhi, protein expression systems based on vaccinia virus and baculovirus have been standardized.

In total, there may not be more than a dozen groups in the country, where recombinant DNA and the associated modern technologies can be applied to molecular biology research with confidence.

The development in the country during the last 5–6 years in the area has been phenomenal and very impressive. This competence has also led to larger investments in biotechnology public sectors. These include the establishment of the ASTRA Research Centre at Bangalore and the opening of the new laboratories of the CCMB at Hyderabad. While, this is a heartening progress in itself, an assessment of the international scene would indicate that India is a decade behind the progress achieved in the West.

3. INTERNATIONAL STATUS

Molecular biology research in the West and Japan has seen a phenomenal growth by taking recourse to recombinant DNA approach. Several eukaryotic genes have been cloned and completely sequenced establishing that the eukaryotic gene sequence is a mosaic and is not colinear with the protein sequence. Many proteins have been expressed in suitable expression systems and obtained in gram quantities. Although, commercial exploitation has been possible only in very few cases, large quantities of proteins for purposes of structural studies and X-ray crystallography have been produced. This would indicate that expression systems (*E. coli*, yeast, vaccinia and baculovirus in particular) have been successfully developed and exploited. This has led to an impressive understanding of the structure of receptors and a clue as to the mechanism of transmembrane signalling. Protein domains necessary for translocation across membranes have been identified. A wealth of information on transcription factors, their mode of interaction with promoter sequences and mechanism of transcription activation has been obtained. New concepts based on the commonality of the mechanisms involved in transcription and replication have been proposed. Detailed molecular models are available to explain DNA repair and recombination in prokaryotes. Molecular parasitology has seen the evolution of some exciting concepts. The molecular mechanisms of the strategies employed by the parasite to escape immunosurveillance are beginning to be understood. A new possibility to use cloned proteins as vaccines to protect against infectious diseases has arisen out of an understanding of the functional importance and strategic location of such proteins in the infecting agent.

The major systems that have contributed a great deal to understand the structure, organization and expression of eukaryotic genes are the immunoglobulin and oncogene families. A virtual knowledge explosion has taken place in these areas and one is getting to be confident about handling autoimmune diseases and cancer. Although AIDS has created a genuine scare, the amount of knowledge accumulated on the molecular biology of HIV is impressive.

Post-transcriptional mechanisms, which include splicing and RNA processing, RNA editing and transport across nuclear membranes have been understood in great detail. The universality of the genetic code has been questioned. The structure of messenger, ribosomal and transfer RNAs and their participation in protein synthesis have been studied with better tools.

Plant molecular biology lagged behind in the beginning, but is soon catching up. A wealth of information has been obtained on bacterial genes fixing nitrogen and the plant genes involved in a symbiotic relationship. The concept of 'biocides', where the plant makes its own insecticide through a toxin gene cloned into the plant holds tremendous promise in understanding heterologous gene expression in plants besides its economic potential in agriculture. The plant system is still faced with the lack of adequate vectors to incorporate foreign DNA. But, Ti plasmids have worked wonders and newer approaches such as electroporation and DNA-coated tungsten bullets to introduce foreign DNA have been efficiently used.

Considerable information on organelle biogenesis and its implication to cell evolution is now available. These studies have implications for improving plant productivity.

The molecular biology of animal viruses; SV40 and adenovirus, in particular, has been studied in great detail and this has laid the foundation for understanding several aspects of eukaryotic gene structure.

4. GAP AREAS

As already indicated, the progress in molecular biology research in the country during 1980–88 is very impressive. But, virtually a knowledge explosion has taken place elsewhere and therefore Indian scientists are lagging behind by nearly a decade.

The main deficiency in research is not lack of ideas or funds, but a lack of the mastery of modern techniques. For example, DNA sequencing and oligonucleotide synthesis have become commonplace in the West. In India, only very few laboratories have this expertise. Gene cloning and characterization are very widely carried out in the West and not necessarily by molecular biologists only. This tool is used by enzymologists, biophysicists and chemists. In India, gene cloning is still considered a great achievement in itself, although in present day molecular biology it has necessarily become the first step. Similarly, many genes have been successfully expressed in appropriate expression systems and large quantities of many proteins have been prepared for physico-chemical studies. India has a long way to go in this area. Even basic methodologies in nucleic acid and protein analysis such as nicktranslation, end-labelling and blot hybridizations pose major challenges to many in the field in India. Elsewhere, these have become so routine as weighing operations on a balance.

The reasons for this situation are clear. These are:

- poor standards of students at the Master's level in universities.
- poor infrastructure for advanced research in universities. This includes non-availability of continuous power supply and water for research.
- necessity to import all equipment and consumables from abroad and the associated agonies involved in the process.
- poor library facilities in universities.
- unfamiliarity of the senior scientists with the modern techniques.
- lack of a critical mass doing high quality research in any particular aspect of the total field. These groups work in isolation within the country, except for contacts abroad.
- a general lack of self-criticism and pride in one's own work and being Indian.

Thus, these reasons are general and should be applicable to all research areas. Perhaps, the one reason specifically applicable to molecular biology is the requirement of highly perishable imported biochemicals and a variety of plastic and other wares. The research is expensive and strictly time-bound. The other reason could be that the tremendous pace set by the industrial infrastructure created by interested parties in the West can in no way be matched by half-hearted enthusiasms shown by entrepreneurs in the country.

5. NEW THRUST AREAS TO BE PROMOTED

The identification of new thrust areas, as already indicated, has to recognize the following factors:

- Recombinant DNA and the associated technologies have become powerful approaches to study molecular biology.
- While there cannot be much of a compromise in the modern techniques to be used, the systems employed can be totally indigenous and relevant to our societal needs.
- Thrust area is only a listing of areas of relevance and importance but need not be exclusive. Therefore, while a certain percentage of funds (to be decided) may be reserved for thrust areas, perhaps a lesser percentage should also be earmarked for non-thrust areas. It should be made clear that in intellectual pursuit, non-thrust area research is as much valuable as thrust area research, although the latter may attract a greater percentage of total funding.

With this philosophy, the following thrust areas are suggested:

Microbial world

5.1 Molelcular biology of tropical pathogens/parasites
    5.1.1 Molecular basis of pathogenicity
    5.1.2 Host-pathogen interaction
    5.1.3 Genetics of pathogens
5.2 Genetic manipulation of microbes for the production of useful metabolites
5.3 Cell and molecular biology of potentially useful microbes
    5.3.1 Mycorrhiza/endophytes, cyanobacteria, *Bacillus*, *Streptomyces*
    5.3.2 Ore leaching bacteria
    5.3.3 Marine microbes
    5.3.4 Microbes tolerant of extreme conditions
    5.3.5 Molecular biology of tropical crop plant viruses
    5.3.6 Insect viruses
    5.3.7 Animal viruses causing disease
5.4 DNA transactions.

Plant kingdom

5.5 Cytoplasmic male sterility
5.6 Organelle genetics
5.7 Differentiation in higher plants
5.8 Stress tolerance in plants
5.9 Genetic transformation in plants
5.10 Plant–microbe interaction
5.11 Gene structure and expression
5.12 DNA transactions

Animal kingdom

5.13 Gene structure and expression
5.14 Molecular basis of development and differentiation.
    5.14.1 Fertility control
    5.14.2 Fetal development
    5.14.3 Post-natal development and differentiation
    5.14.4 Molecular basis of immunity
5.15 Molecular mechanism of transmembrane signalling
5.16 Molecular basis of sex determination
5.17 Gene transformation in animal cells and embryos
5.18 DNA transactions

6. HUMAN RESOURCES DEVELOPMENT

As already indicated, our major concern is the availability of competent manpower. In the light of the largeness of our country and the number and dimensions of problems that can be solved through modern biology and the total amount of scientific manpower available in the country, the lack of a critical mass of competent research groups in the area only reflects a distorted emphasis on manpower development. The often boasted large scientific manpower in the country is actually of inferior quality. The fundamental issue between spread of education among the masses and achieving peaks of excellence has to be solved at the university level. Higher education at the college level has to be exclusively meant for gifted students. While general education should be assured to all segments of society up to say 12th standard, further collegiate education has to be made exclusively on the basis of merit. This is a larger issue and cannot be treated in this document adequately. However, this is a fundamental issue to be kept in mind in terms of approaching the problem of human resource development in any advanced area of science and research.

In the limited context of human resource development in the area of molecular biology the following factors should be recognized:

6.1 Many academic and scientific posts in the area in the country are lying vacant due to the non-availability of suitable candidates.

6.2 The standard of teaching of modern biology in universities is poor, in view of the unfamiliarity of the existing teaching community with current progress in the field.

6.3 Research and laboratory training in the field are of very poor standard, again due to the unfamiliarity of the senior faculty with the present day molecular biology and lack of infrastructure.

6.4 Many state universities have started molecular biology or even biotechnology without adequate preparation in terms of having appropriate faculty and laboratory.

6.5 Bulk of the experimental research done even in National Laboratories and some of the outstanding universities is still only the second best, while the country has always produced at least a few outstanding theoretical groups. This only emphasizes our deficiency in the experimental approach.

Some of the limited and short range solutions to the problem are:

- DST should pool resources with other agencies and start new centres for research in molecular biology in universities in the country. These centres should be built around identified scientists. These centres should be built in not so well known universities, which may still have at least one or two good scientists. An honest search needs be undertaken to identify such individuals and groups, who may not be in the limelight.

- Industries should come forward not only to sponsor research projects in universities, but also fund establishment of new research centres in the university set-up. The universities should receive such proposals with an open mind and facilitate the establishment of such a centre. Competent scientists in industrial set-ups should hold honorary faculty positions in universities.

- Adequate support should be given by the DST to the existing centres/groups that have performed well.

- DST can initiate training associateship programme for scientists in service. There can be two categories one for training within the country and another for training abroad. This scheme appears to be working reasonably well with the Department of Biotechnology.

- Despite the enormous brain drain, still adequate number of well trained Indians have shown interest to return and settle down back home. This group usually consists of young scientists who have spent around 3–5 years abroad. This is also the group most ideal to have back in India. It is necessary for universities to have a mechanism to offer jobs to candidates with good credentials on a priority basis. It should be possible to make a decision within 3 months time. The present procedures adopted in many universities are very slow and good candidates are lost. The second major factor is to create an environment congenial to retain the new comers. This can only be provided with good academic leadership and adequate infrastructure.

- Molecular biology teaching and laboratory at M.Sc. level should be strengthened.

7. NATIONAL FACILITIES REQUIRED

Human resource and infrastructure are essential for progress in research. The subject of human resource development in a limited context has been dealt with earlier. The following suggestions are for infrastructure development:

- The COSIST programme has brought about a qualitative change in research infrastructure in some universities. This programme should be expanded and many more centres and groups should be identified and strengthened.

- Some steps have been taken to liberalize import procedures. It has to be really seen how well the recent liberalizations are implemented. Many times, there is a difference between intention and what is actually implemented in the field.

- Manufacture of biochemicals and research instruments should take place in the country. Best course appears to be to encourage collaborative ventures between Indian and foreign companies, so that the product is manufactured in India.

- The Department of Biotechnology has created several national facilities such as microbial collection centre, centres for animal and plant tissue cultures, bioinformatics and is in the process of creating national facilities for oligonucleotide and peptide synthesis. These facilities will have a direct impact on the quality of molecular biology research in the country. It has to be seen as to how well these national facilities are able to contribute to the growth of molecular biology in the country. Everything should be done to facilitate the optimum performance of these national facilities.

8. OTHER SUGGESTIONS

The following general issues also need to be considered.

8.1 Funding by research agencies in isolation is a serious lacuna. Many agencies are funding similar areas of research. Integration has been talked about, but very little has been done in practise. In the field of molecular biology, a beginning can be made by integrating the efforts of the Department of Biotechnology and DST to start with, perhaps, projects, specialized centres/units, training programmes, Summer/Winter schools, etc. and then a decision made as to which of the two agencies is most appropriate to fund the proposal. In fact, the entire life science programme has to be an integrated effort between DBT and DST, while keeping the overall objectives of the two departments in perspective.

8.2 The PAC mechanism followed at the DST evaluates new proposals and offers mid-course correction to on-going projects. However, no serious analysis and gradation of completed projects take place. This should be made mandatory and the scores obtained in completed projects, should contribute to the decision making process for funding new proposals.

8.3 There is no common evaluation procedure adopted by different PACs. A quantitative scoring procedure should be evolved and decisions made on the basis of the scores obtained by the proposals.

8.4 The large opportunities available at the present time to go abroad for training etc. have led to good and bad results. In many laboratories, even competent scientists tend to carry out the work abroad but do not achieve very much back home and keep waiting for the next trip abroad. While, training in established laboratories in the West is a welcome feature, ultimately Indian science can grow only if things start working in Indian laboratories. Therefore, serious attempts at technology transfer should be made by the individuals who benefit by the exposure. Thus, some kind of monitoring of the laboratories receiving substantial funding from DST is essential. This should include site visits, both for assessing what has been achieved and for sanctioning new major projects, to evaluate the technical competence developed in the laboratories concerned.

MOLECULAR BIOPHYSICS AND BIOCHEMISTRY

1. PREAMBLE

MOLECULAR biophysics and biochemistry forms the foundation for the fundamental and technological advances in the areas of molecular biology, bio-technology and medical physics. Both fields have grown at an extremely fast rate during the past 50 years. The fundamental knowledge about the basis of molecular mechanisms involved in biology started emerging from 1940s and got accelerated with the discovery of molecular structure of proteins and nucleic acids. Our country has picked up these areas at an early stage and the contributions made by Indian scientists in this field are of a very significant nature.

While formulating the thrust area programmes in the two disciplines of biophysics and biochemistry during the Baroda meetings, the activities were divided into various Programme Advisory Committees under the disciplines of physics, chemistry and biology. DST has realized in recent years that the fields of biophysics and biochemistry are intimately connected and such a division of activity may not be advisable in the interest of promoting integrated and interdisciplinary programmes in these overlapping areas. DST therefore took the conscious decision to combine these activities under a common thrust area programme.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

During the VI and VII Plan Periods, the emphasis of the research programmes had been on enzyme structure and function and conformation of biological molecules. When the VI Plan activities were initiated under the thrust area programme there were very few major facilities to support these activities. Much of the earlier research output was therefore based on good ideas backed by primitive facilities and we were loosing the race with the world. Therefore, the PACs made attempts to create sophisticated facilities and to make Indian scientists aware about the recent trends in these areas through workshops, winter schools, etc. The twin areas received good funding during this period. Money was provided for creating state-of-the-art X-ray facilities at IISc, Bangalore and at the University of Madras, Madras. Another good X-ray diffraction facility for macromolecular study exists at BARC, Bombay which has been built up largely through indigenous efforts. DST also provided funds for creating good NMR facilities at TIFR, Bombay; IISc, Bangalore; CDRI, Lucknow and Bose Institute, Calcutta. For biochemical work, individual researchers were funded so that the basic facilities such as HPLC, centrifuges, etc. are now available in several laboratories. Facilities for tailored DNA and peptide sequences were also made available. In view of the complex nature of these facilities, most of the inputs were made in institutions with good back-up and infrastructural facilities. At the same time, as a result of these investments, we now have expertize and facilities which can be utilized by a more general group of users located in the universities. In particular, it is now possible at least in principal, that small programmes can be funded in the universities without providing major grants for sophisticated instruments and biochemical facilities. As an approach to the VIII Plan, the PAC must encourage such researchers. DST should also ensure that the facilities funded with its money are shared with the users all over the country who have viable research programmes.

3. GAP AREAS

The field of biophysics and biochemistry has blossomed into several new areas which now lie on interface with the emerging trends in biotechnology and bioengineering. Taking this in view, the PAC felt that a major change is required in the structure of programmes which had been identified as thrust areas several years back. PAC also felt that brain storming sessions should be held in the area of protein engineering and protein–DNA interactions. Scientists have been identified to take initiative to organize such sessions. Areas such as biomolecular electronics and protein designing may provide focal themes for future research programmes.

A new interface is developing involving molecular techniques, artificial intelligence and medicine and such areas of medical physics should be encouraged. Examples of the type of problems one is referring to are molecular mechanisms involved in the process of sleep, fatigue, pain, animal behaviour, learning, etc. Such studies have been made possible because of recent developments for monitoring molecular levels through *in vivo* techniques and image reconstruction. Research in these fields requires major investments and bringing together expertize in the fields of molecular, computer and medical sciences working towards a common goal. Since such an area cannot be developed under the present scheme of TAP (funding to individual scientists or a group of collaborators on a fixed time basis), it has been included as a separate item in the list. It is an ideal subject to be looked into by DST and ICMR under the scheme of Intensification of Research in High Priority Areas. The inputs made at this stage can put us in the forefront of this field and can bring a new revolution in health care as well as development in future design of computers based on information gained from nonlinear neural networks.

We can take pride in the fact that in certain areas our research is on the frontline and that we are running the race with the world. However, we should not forget that in most cases we are following the science initiated by scientists in western countries. Even in cases where ideas originate in our own country we soon loose the race because the ideas are picked up by scientists with better resources. One of the things that is lacking in our research is an interdisciplinary approach and sharing of our limited resources and expertize to achieve a concerted and common goal. The areas under discussion require knowledge at the level of physics, chemistry, biology, computer sciences and mathematics. It will be difficult to make a dent unless Indian scientists with various strength can get together and work towards common goals.

4. NEW THRUST AREAS

4.1 Membranes and model systems
4.2 Bioenergetics
4.3 Biochemical evolution
4.4 Molecular structure, interactions and recognition in biological systems
4.5 Protein engineering: (site-direct mutagenesis, cloning and designer proteins)

4.6 Biomolecular electronics
4.7 Catalytic antibodies
4.8 Enzyme structure and function
4.9 Molecular mechanics and dynamics
4.10 Molecular mechanisms of perception and communicaiton (chemoreceptions, cell surface recognition, drug receptor interaction).

5. Important area which may be supported under IRHPA.

Medical physics (details as outlined above).

PLANT SCIENCES

PREAMBLE

As primary producers of renewable resources that provide the basic needs of humans and domestic animals and a variety of raw materials for industry, green plants will continue to be pivotal for our civilization. The full potential of plants and microbes has only been marginally realized. Basic scientific knowledge and its application will have to be pressed into service effectively to mitigate poverty, meet the country's requirements of food, feed, clothing, rural energy and also sustain the rich plant diversity, prevent eco-degradation, recover wastelands and ensure biological conservation. The well-established methods of genetic improvement of plants and microbes need to be speeded by the application of genetic engineering and biotechnology.

A perusal of the Thrust Area Programmes in Life Sciences emanating from the Baroda Seminar shows that it did not identify any aspect of Plant Sciences including microbiology in the area of basic Life Sciences.

However, the following areas were recognized under Applied Biological Sciences.

- Microbial productivity
- Microbial genetics
- Improved strains for fermentation
- Microbial transformation
- Useful biochemicals and micro-organisms
- Biomass as a source of energy
- Fast growing plants
- Biological fuel cells
- Biogas
- Production of alcohol
- Physiology and biochemistry of plants
- Photosynthesis (including photorespiration)
- Nitrogen fixation
- Improved plant proteins
- Protection of endangered species and preservation of genetic diversity of living organisms
- Ecological balance for sustainable utilization of biological resources
- Forests
- Grazing lands
- Cell and tissue culture
- Somatic cell hybridization.

In this list, those areas which pertained to environment were transferred to the newly created Department of Environment.

The programme advisory committee reiterates that excellent and innovative basic research in plant and microbial sciences must be supported by DST in keeping with its past tradition. Nevertheless, certain areas of work which are either relevant to national needs or would fill the present lacunae must be recognized as thrust areas.

In some of the thrust areas multidisciplinary groups should be organized at three or four centres, which should have the potential to become leading research groups in the world. Research in areas such as DNA sequencing in plant mitochondria, chloroplasts and nuclear fragments and study of stress due to water, disease and salt is of such magnitude that a small group working in isolation can make very little progress. It is essential to have collaboration of specialists in biochemistry, membrane biology, plant genetics and rDNA technology. Three or four thrust areas and a few research groups should be identified and a long term (5–10 years) programme initiated. The work of the different centres may be reviewed periodically and the programmes suitably revised in the light of recent developments. Interaction with foreign groups may also be needed in some areas. DST has provision for supporting units and core groups. However, thrust should be given to interdisciplinary and inter-institutional research on a long term basis. Whereas good work is being done by a few research groups working in isolation, the results have not been commensurate with the talents and facilities available in the country.

It is to be recognized that plant scientists have had a long and rich tradition of research in this country and that they constitute a very large proportion of biologists in India. The list of thrust areas may appear long but it is realistic, considering their basic and applied value in the national context.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

2.1 *Photosynthesis*

Extensive work has already been done globally on photosynthesis at the whole plant level. The biochemistry of photosynthesis, environmental effects on photosynthetic carbon dioxide fixation, the relationship between the electron transport capacity and carbon dioxide assimilation rates have been extensively studied. However, it is still obscure how the primary processes in photosynthesis which are ultra fast reactions in the range of femto to pico seconds are controlled. Studies on the genetic regulation of these events by the modification of structure of photosynthetic apparatus is now basically required to be studied.

The conversion of light energy into chemical energy during photosynthesis can be fully understood only when the molecular architecture of the photosynthetic pigment molecules in the membrane is known. The energy absorbed by the pigment proteins is efficiently transferred to the reaction centres of either photosystem I or photosystem II. These multimolecular complexes have been partially characterized by their structural and functional association within a specific photosystem. It is still not clear how the isolated chlorophyll protein complexes are connected with the LHCs *in vivo* to produce photosynthetic units. We do not yet know how many apoprotein molecules are assembled with each other (and with chlorophyll *a* and *b*) to form the LHCs in the thylakoid membranes. Though the structure of pigment complexes in green plants has been moderately established (particularly of LHC II), no crystals suitable for high resolution X-ray analysis have been reported so far for any plant pigment-protein. The oxygen evolving complex has not been totally isolated and its arrangement in the membrane has not yet been delineated.

2.2 *Nitrogen fixation*

Various aspects of nitrogen fixation have been studied at the Bhabha Atomic Research Centre, Banaras Hindu University and a few other institutes. Work done at BARC has shown that: (i) heterocysts lack phycobiliprotein and PS-II activity; (ii) heterocyst development correlates with nitrogenase activity; (iii) heterocyst is the main site of nitrogen fixation; (iv) sodium is required for nitrogenase activity but not for its synthesis; (v) prolonged incubation of cultures with acetylene brings about conformational changes in the nitrogenase polypeptid; and (vi) glutamine synthetase and glutamate synthetase are the main ammonia assimilating enzymes in cyanobacteria.

Recently, scientists at BARC have successfully cloned *nif* genes both from heterocystous and non-heterocystous cyanobacteria. The major contributions from BHU and Hyderabad have been in understanding the mechanism governing heterocyst differentiation and nitrogenase synthesis. Ammonia *per se* is not the regulator of heterocyst differentiation or nitrogenase activity. Employing amino acid analogues and *nif* mutants it has been shown that glutamine regulates heterocyst differentiation. Glutamine is also involved in maintaining the spacing pattern of heterocysts. Possible linkage between nitrate reductase and nitrogenase has also been suggested. The effects of various physical and chemical factors (e.g. herbicides, nitrogenous fertilizers, growth inhibitors) have been studied on diverse nitrogen fixing cyanobacteria. The symbiotic cyanobacterium *Anabaena azollae* has been isolated and it has been suggested that both morphological and physiological changes occur in the symbiont when it is freed from the host.

Blue-green algae are the only organisms in which the otherwise incompatible processes of nitrogen fixation and photosynthesis occur together in the same filaments.

2.3 *Tissue culture*

Plant tissue culture research in India was initiated 30 years ago primarily with the objective of using it as a technique for understanding problems related to reproduction in flowering plants. India has carved for itself a place in the international sphere for its basic contributions to *in vitro* pollination and fertilization, production of androgenic haploids and triploid, etc.

Work done in India has stimulated research in several laboratories around the world although in our own country, we have not been able to realize its potential optimally. However, in the past decade there are indications of taking the technique to the level of technology especially in the micropropagation of elite economic plants such as oil-seed crops, legumes, timber and fire wood crops and medicinal and aromatic plants.

One of the main reasons for the slow pace of progress in the full utilization of plant tissue culture is lack of orchestration and interdisciplinary approach to solving specific goal-oriented problems. The seed money grants made to several individuals and groups have resulted in research activities that have given us the confidence to make important dents in agriculture and forestry. The country has produced a critical mass of trained human power to take up the challenges.

2.4 *Microbial productivity*

The country has not made sufficient progress in microbial productivity either in developing efficient microbes or in improving the engineering. Microbial productivity in India is largely centred on production of antibiotics and yeast. Even in these, our yields are below the international standards.

Projects in the thrust area were few and have been unsatisfactory. Thus in this most important area funding has been negligible.

A critical review of the national industries in this area has shown that there is a tendency to maintain *status quo* in terms of production or import of improved strains and adopt them to out dated machinery. This has failed to increase productivity. Bulk production may have gone up, but the unit input cost has not come down.

3. NEW THRUST AREAS

3.1 Reproductive biology
3.2 Differentiation, development and hormones
3.3 Genetics and plant molecular biology
3.4 Biology of stress
3.5 Photosynthesis and photobiology
3.6 Biology of trees
3.7 Plant pathology
3.8 Microbiology.

3.1 *Reproductive biology*

Indian scientists have made significant contributions to descriptive, comparative and phylogenetic aspects of plant embryology. In fact they can be considered as leaders in the world literature in these areas. However, in terms of basic knowledge needed for improvement, utilization and conservation of plants of high economic value, our efforts have been rather minimal. Recognizing the expertize already available within the country, the PAC recommends that the following aspects may be supported:

3.1.1 Pollination biology, especially of forestry species and other economically valuable perennials

3.1.2 Pollen–pistil recognition and interaction

3.1.3 Isolation of male gametes of flowering plants and their use as tools in biotechnology

3.1.4 Regeneration of isolated plant cells and protoplasts following genetic transformation

3.1.5 Molecular and biochemical mechanisms of male sterility, apomixis and somatic embryogenesis

3.1.6 Storage of pollen as gene banks

3.1.7 Biology of seed set, biochemical aspects of seed protein storage, seed viability and germination

3.1.8 Production of artificial seeds (with *Rhizobia* and/or mycorrhizae along with somatic embryos encapsulated with hydrophobic coating)

3.1.9 *In vitro* preservation of germplasm especially of shoot tips or somatic embryos of economic plants with recalcitrant seeds.

3.2 *Differentiation, development and hormones*

The development in multicellular plants involves the formation of several distinct cell types, tissues and organs in a precise time-phased manner at specific locations. The cells of the apical meristems are known to retain the embryonic nature permanently. However, in many plants even the fully differentiated cells upon culture in an artificial medium can be made to divide and develop into whole plants. This reversibility of commitment makes it possible to regenerate and multiply plants through tissue culture and other means. Such systems are also invaluable for understanding the basic features of plant cell differentiation. The overall plant development is regulated by hormones, nutrients, environmental factors such as light and temperature.

The following areas need thrust:

3.2.1 Analysis of cell lineages, developmental commitments and role of genes in development

3.2.2 Hormonal regulation of development and differentiation, temporal and spatial variations in hormones, signal transduction during hormone action, regulation of transcription and translation. Role of calcium.

3.3 *Genetics and plant molecular biology*

Plant genetics and breeding have contributed a great deal towards making the country self-sufficient in food production; increasing export of agricultural produce or products of agro-based industries and contributing to the rural economy.

The overall contribution of genetics to the national economy is far greater than that of any other discipline in plant sciences. Increased investment in research in this discipline can bring even greater returns.

The promise and potential of plant biotechnology is based on the use of cell culture and molecular biology techniques to produce plants that have enhanced consumer (such as improved nutrition) or value added agronomic qualities. These accomplishments have been achieved not in major crop species but in model systems such as *Petunia*, tobacco, tomato, etc. which are easy to manipulate.

The first products of biotechnology concern viruses and bacteria. Unfortunately, no major crop species or plant products improved or produced by biotechnology are yet available.

The following thrust areas are identified:

3.3.1 Genome organization of a few selected cereals, pulses, oil seeds and forest trees

3.3.2 Identification, cloning, horizontal transfer, regulation and expression of useful genes

3.3.3 Genetic and molecular basis of compatible (susceptible) and incompatible (resistant) interactions between plants and pathogens, insects and symbionts

3.3.4 Genetic and molecular basis of resistance to abiotic stresses.

3.4 *Biology of stress*

With increasing human and live stock population, there is an ever increasing demand to produce more on limited cultivable land area as well as to utilize wastelands. In addition to the need to understand how crops adapt themselves to low moisture, flooding, high salinity and other soil stresses, there is an urgent need to probe into the mechanism of tolerance to heat, cold, salinity, alkalinity, etc. Special attention needs to be given to:

3.4.1 Molecular mechanisms that regulate water and salt stress

3.4.2 Adaptation to salinity and alkalinity

3.4.3 Isolation and utilization of *Rhizobia* tolerant to stresses

3.4.4 Genotypic responses and screening for water stress in pulses, oil-seeds and tree species.

3.5 *Photosynthesis and photobiology*

Photobiology is an interdisciplinary science which has undergone remarkable advancement during the past few years. The chemical evolution of the light absorbing pigments such as chlorophylls and carotenoids has led to the harvesting of solar energy and transforming it into chemical energy. Photosynthesis results in the production of high energy organic compounds (biomass) from low energy precursors. The photoautotrophic organisms have an ecological advantage over chemosynthetic organisms since the solar energy is available in a virtually unlimited supply. In addition to energy fixation, plants use light for a wide range of complex sensory processes. The necessity for photo orientation is obvious in photosynthetic organisms which depend on the availability of radiation.

The following areas in photobiology require thrust:

3.5.1 Isolation and sequencing of genes encoding PS subunits

3.5.2 Elucidation of the three-dimensional structure of the functional macromolecular complexes in the thylakoids of the photosynthetic apparatus

3.5.3 Genetic engineering of chloroplasts (chloroplast transformation) for improving yields and conferring herbicide resistance

3.5.4 Study of unclear-coded genes for chloroplast polypeptides

3.5.5 Understanding of the regulation of activity of RUBP carboxylase

3.5.6 Steric configuration of phytochrome

3.5.7 Molecular basis of photomorphogenesis

3.5.8 Photomorphogenesis of mitochondria.

3.6 *Biology of trees*

Tree biology research useful for afforestation is important to meet the needs of national programmes of social forestry, timber production, wasteland afforestation, water-shed afforestation and fuelwood and fodder production. Although India possesses an array of tree species uniquely useful for different purposes, we have very little information on their biology. As in other parts of the world, India can benefit enormously by modern approaches for improving, identifying, modifying and regulating tree species.

The following areas need support:

3.6.1 Forest genetics and tree improvement

a) Screening of genetic variation of wild tree species
b) Identification of chromosome races and multiplication of triploid trees for fast growth and higher productivity
c) Selection of provenances, race and plus trees to be subjected to site-performance evaluation, followed by progeny tests and advanced generation, selective breeding and hybridization
d) Reproductive biology of selected trees
e) Broadening of the genetic base through indigenous and exotic material and evolving procedures for utilization of hybrid vigour.

3.6.2 Tree physiology

a) Physiological aspects of juvenility and maturity
b) Flowering, fruiting and seed development
c) Seed viability, dormancy and germination
d) Storage of recalcitrant seeds
e) Vegetative propagation with special reference to hard-to-root plants
f) Micropropagation of elite trees
g) Photosynthesis and biomass production
h) Growth under stress conditions with special reference to physiology of roots
i) Nitrogen fixation in trees in symbiotic association with rhizobia and actinomycetes
j) Mechanism of mycorrhizal-tree association and its economic utilization
k) Ultra-structural and physiological aspects of cambial activity, wood and bark production
l) Enhancement of secondary metabolite production—especially rubber, gums and resins by growth regulator application.

3.6.3 Tree pathology and protection

3.6.4 Application of biotechnology and tree improvement.

3.7 *Plant pathology*

The basic and applied aspects of plant pathogen interactions have received much attention by scientists the world over. For a predominently agricultural country such as India, plant protection is crucial in crop productivity.

Biological control of plant pathogens is an area in which very little work has been done in the country. The technologies developed in the recent years have demonstrated good potential for the biological control of bacterial and fungal diseases. Programmes on the biological control of important diseases should be strengthened.

In the area of physiological plant pathology, limited information is available on the processes of infection, role of various enzymes and defence mechanisms in plants in relation to plant diseases common in India. These studies require close collaboration among pathologists, physiologists, geneticists and biochemists.

A few aspects which need urgent attention are summarized below:

3.7.1 Plant–microbe–viral interactions

3.7.2 Associative symbiosis for enhanced plant growth (*Frankia*), Pseudomonads, etc.

3.7.3 Biological pest control (excluding work on *Bacillus thuringensis* toxin)—Emphasis on baculoviruses, fungal pathogens of pests, pheromones.

3.7.4 Monoclonal antibodies for detecting, monitoring and effective management of plant diseases.

3.8 *Microbiology*

Realizing that five years from now genetically engineered strains could be used for microbial productivity, it is important to support research in microbial biomass. A complete new strategy has to be worked out. A dialogue with industry will be needed.

The following areas need thrust:

3.8.1 Plant growth regulators of microbial origin

3.8.2 Enzymatic processes for chemical technology reactions and newer process developments (e.g. indigo, arachidonic acid, etc.)

3.8.3 Enzymes for detoxification of major chemical pollutants, pesticides, insecticides, etc.

3.8.4 Enzymes for paper, leather and other commodity industry applications

3.8.5 Enzyme reactions in organic solvents

3.8.6 Synthetic enzymes

3.8.7 Enzymatic, immunological and recombinant DNA methods for disease diagnosis-clinical enzymes and diagnostic kits.

3.8.8 Screening for anti-inflammatory, anti-cholesteremic, anti-parasitic compounds from microbes, new immunosuppressants, etc.

4. HUMAN RESOURCES DEVELOPMENT

4.1 Training and refresher courses in the following areas may be held periodically:

4.1.1 Plant molecular biology and genetic engineering

4.1.2 Plant protoplast isolation, regeneration and transformation

4.1.3 Use of computer software for DNA and protein studies, literature retrieval and statistical analyses

4.1.4 Techniques of plant virology

4.1.5 Environmental toxicology

4.1.6 Environmental impact analyses.

4.2 The syllabi for plant sciences require revision to incorporate recent advances. A committee of experts may be formed in collaboration with UGC to revise the syllabi.

4.3 An integrated B-Tech. programme in microbial engineering (4 years duration after 10+2 years of schooling) must be started.

5. NATIONAL FACILITIES REQUIRED

The following national facilities are needed for rapid progress in several areas of plant sciences:

5.1 Plant hormone assays: A national facility for assay of phytohormones (auxins, cytokinins, gibberellic acid, abscisic acid, ethylene) is required. These hormones play a basic role in cell growth and differentiation and in several cell processes. Sensitive methods based on radio-immunoassay or Elisa, HPLC and gas chromotography are used for their determination which are not available to most research workers. A national facility where the analyses of these hormones can be undertaken will be invaluable.

5.2 Collection and assay of plant viruses: The number of plant viruses of economic importance is very large. A centre for maintaining a collection of viruses and sera banks and monoclonal antibodies for virus assays would greatly extend the scope of research in this important area.

5.3 Controlled environment growth chambers: At many centres of research, work on a particular plant species is seasonal and progress is, therefore, very slow. The availability of controlled environment growth chambers would facilitate the growth of these species throughout the year. The imported growth chambers are very expensive. Research on indigenous production of these chambers should receive high priority. A committee may be formed to make recommendations for planning indigenous production of these chambers.

5.4 Chemicals, enzymes and equipment for research: Several special biochemicals, restriction endonucleases and other enzymes and equipment such as millipore filters, etc. are needed for research. They are presently imported through the CSIR Centre for Biochemicals, but indigenous production and ready availability of these items will be very useful for research.

5.5 Computer software: Research on DNA and protein structure and statistical analyses require software for which DBT is establishing a national network. The provision of computers to different research groups for integration into the network for literature and for use of the software is required.

5.6 Long term ecological studies: At least one site in each biome including agricultural ecosystems, should be made available for long term biological studies. They should be equipped with a field laboratory and skeleton staff.

5.7 Microelement analysis: A national facility equipped with atomic absorption spectrometer/X-ray fluorescence analyser for rapid determination of micronutrients is needed for many projects. Though there are a few centres which carry out these analyses, especially on elements such as iron, molybdenum, manganese and zinc, there are often long delays in carrying out these analyses. Facilities for analysis of other elements such as cobalt, nickel, selenium and fluoride are also needed.

5.8 A national laboratory entrusted with the exclusive study of antibiotic producing strains must be set up. For another 50 years to come microbial antibiotics will be still needed in India inspite of the availability of antibiotics and synthesis.

ANIMAL BEHAVIOUR, ECOLOGY AND EVOLUTION

1. PREAMBLE

ETHOLOGY, the scientific study of animal behaviour, is relatively a recent field of animal sciences integrating animal ecology, neurophysiology endocrinology, sensory physiology and several other fields. Behavioural studies, in the country, have been essentially confined to only a few animal groups in spite of the fact that we in India have the great fortune of having access to a tremendous diversity of plants and animals, notable among which are many groups of insects, birds, primates and human societies which are uniquely suited for modern investigations in ethology and sociobiology. This fact is widely recognized in our country, but this resource is hardly utilized. On the other hand there is a great deal of interest among western scientists to come to India to take advantage of these valuable biological materials. Given a well planned programme, we have an excellent chance of providing international leadership in this area.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

Behavioural studies in this country have been essentially confined to such principal areas as primatology involving non-human primate behaviour, behavioural dynamics of rodents, migratory behaviour of birds as well as reproductive behaviour of some fishes in relation to endocrine control of reproduction. In addition, in recent years some work has been done on circadian rhythms in bats and to a limited degree on the chelonian and crocodilian behaviour. Compared to information on the behaviour of vertebrates, that on invertebrates is restricted mostly to arthropods. An outline of the current status of research on ecobehaviour and evolution is indicated below.

2.1 Behavioural studies

2.1.1 Vertebrates

– Behavioural ecology of wildlife

Investigations in this particular field have been carried out on such animals as elephant, rhinoceros, lion, tiger, antelopes, etc. with reference to their territorial behaviour, predation, parental care, defensive behaviour, etc. The newly-established Wildlife Institute of India (Dehra Dun), has come up with excellent programmes to study the behaviour and ecology of several species in relation to their habitat.

– Behaviour of non-human primates

Studies relating to the behaviour of non-human primates including their sociobiological aspects, territoriality and parental care have been extensively carried out by such centres as the Zoological Survey of India, the University of Jodhpur (Department of Anthropology), Delhi University, Aligarh Muslim University, Meerut University, and at a number of other centres.

– Rodent behaviour

Studies on the socio-ecology of desert rodents are in vogue for the last 30 years at the Central Arid Zone Research Institute (Jodhpur), and on rodents of economic importance to agriculture at 10 centres in India under the ICAR All India Coordinated Project on Rodent Pests. Some work was taken up on the ecology and reproductive behaviour of murids under a DST-funded project at the Garhwal University. Besides considerable work on the ecology of bandicoots and other species has been carried out at the Zoological Survey of India; Haffkine Institute (Bombay), Department of Entomology (Kanpur). Besides, relatively a new field of ethology, the olfactory communication among rodents, is being carried out at BHU (Varanasi), CAZRI (Jodhpur) and at University of Kerala (Trivandrum).

– Circadian rhythm in bats

Much attention has been paid in recent years to the study of the circadian rhythms in bats by the Madurai Kamaraj University (Madurai).

– Behaviour of birds

Extensive investigations have been carried out on the migratory behaviour of birds, their nesting behaviour, parental care, feeding behaviour by the scientists of the Bombay Natural History Society and the Zoological Survey of India, and that of several universities.

– Chelonians and crocodilians

Investigations on the egg laying, feeding, parental care, and captive breeding of chelonians and crocodilians have been made by some of the university centres and Wildlife Institute of India.

- Fishes

The reproductive behaviour of fishes has been investigated in relation to their schooling behaviour, spawning and parental care, by the Inland Fisheries Research Institute, Centre of Advance Studies in Marine Biology (Portonovo), besides other universities.

2.1.2 Invertebrates

The various aspects of invertebrate behaviour investigated in this country are broadly outlined.

- Helminths
- Behavioural ecology of helminths
- Annelids
- Swarming behaviour in polychaetes
- Earthworm behaviour in relation to vermiculture
- Arthropods
- Crustaceans: Crustacean behaviour in relation to their feeding and reproduction, tidal cycles and lunar rhythms of ecology of crustacean parasites.
- Arachids: Behaviour of ticks and mites in relation to their vector potential, web spinning behaviour in spiders
- Molluscs: Oyster, mussel and clam culture and setting behaviour
- Insects.

(i) Feeding behaviour of phytophagous, predatory and parasitic insects

- Host searching and host selection behaviour
- Feeding rhythm of larval and adult insects
- Feeding behaviour of mosquitoes in relation to disease eradication programmes
- Behaviour of parasitoids in relation to host selection

(ii) Distributional pattern

- Vertical horizontal distribution, behavioural ecology of insect communities
- Sociobiological aspects of Hymenoptera

(iii) Courtship behaviour

- Endocrine control of reproductive behaviour.

(iv) Foraging behaviour of pollinators. Energy expenditure in foraging and the role of behavioural studies in pollination.

3. INTERNATIONAL STATUS

4. GAP AREAS

4.1 Biosystematics: Expertize in taxonomy/biosystematics of certain group of taxa is extremely limited within the country specially reckoning the great diversity of fauna and flora occurring in the Indian sub-continent. Researches in biosystematics need to be strengthened, a database should be prepared on the information already available but scattered and therefore, not useful in the decision making process. Attempts should be made to create a National Identification Centre with special reference to insects.

4.2 Behavioural models: Studies on diverse aspects of animal behaviour, ecology and evolutionary aspects have assumed interdisciplinary proportions calling for indepth investigations. From the point of view of animal behaviour, of several emerging areas of considerable relevance some are behavioural rhythms in various animals and plants, the formulation of flow chart or behavioural models of several species of economic importance more particularly among insects, integrated approaches to behaviour through behavioural manipulation as well as through neurophysiological and neurochemical studies. From an ecological angle diverse aspects dealing with mountain ecology, insect–plant interactions in terms of chemical ecology as well as energy flow in ecosystems populations as well as through the individual life cycles, are emerging areas for taking up research. Needless to mention, the reptiles and mammals of north-east India deserve indepth studies from an ecobehavioural viewpoint.

4.3 Evolutionary approach: Evolutionary approaches are interlinked with ecobehavioural studies and an understanding of the various adaptive strategies adopted by diverse groups of organisms in their efforts for survival go a long way in our understanding of this integrated area of research. In this connection, the evolution of reproduction in animals and plants in terms of the photoperiodic and hormonal basis as well as the evolution of feeding behaviour, more particularly in animals with varying habits such as evolution of predation, evolution of parasitism, etc. are areas wherein more information is needed. The interlinking of ecology with physiology (ecophysiology) and ecology with behaviour (ecobehaviour) further highlights the need for investigations on such areas as stress physiology, neurotoxicology and reproductive physiology.

4.4 Marine science: There is a plethora of ecologically interesting, physiologically significant, economically important and potentially useful organisms in the marine environment. Although the detailed study of these several forms will take a very long time, there are some areas in which research can be encouraged right away so that benefits may accrue in another 5–10 years. Among these areas some of the most rewarding are also the most basic. For example, culture of marine forms has never really been developed in this country. Although ecological statements have been made on trophic level interactions, the dietary habits of the several herbivores and carnivores have not been systematically studied. Neither grazing rates nor respiration rates can be measured unless the species is in monospecific culture. Similarly prey recognition, specially for species that are raptorial feeders or avoidance/rejection by filter feeders have not been investigated. Conditions that control spawning have been deduced from environmental studies, they have not been meticulously investigated in controlled conditions. All of these studies require to be done before attempting ecologically valid predictive models.

4.5 Soil biology and fertility: At a general level this project envisages comparative analysis of natural and man-made agroecosystems at a given site with a view to understand: (a) nutrient cycling patterns, (b) nutrient budget in biomass and soils, (c) litter decomposition patterns through soil animals and soil micro-organisms and even (d) soil fertility recovery during succession if habitat types are available. The objective has to incorporate biological processes into soil fertility maintenance in agroecosystems. This may take the form of mixed cropping, agroforestry concepts, earthworm culture being incorporated on the basis of studies on natural systems.

At a specific level the study may involve field studies on biological nitrogen fixation, earthworm biology and nutrient cycling through vermiculture, potassium or phosphorus fertility maintenance, etc.

This is an area relevant to agriculture and yet is not a concern of agricultural scientists. Nutrient release on surface soil layers through no-till agriculture which is being investigated now, for e.g. imitates natural systems in terms of nutrient release on the surface soil through litter fall.

4.6 Ecology of biological invasion: A variety of questions could be asked related to biological invasion by exotics in our terrestrial and freshwater ecosystems.

- What are the factors contributing to invasion?
- Could invasion occur in intact ecosystems or perturbations critical for invasion to occur?
- What are the ecosystem level consequences at the structural and functional levels of the ecosystem?
- What are the social and biological impacts of invasion?
- What are the social and biological consequences of possible control measures?

The answer to these questions and many others is important for managing our natural ecosystems that are being more and more altered by biological invasions altering biological diversity and even affecting productivity of our land and water resources.

4.7 Ecology of succession: Studies on the ecological succession on a variety of habitats like coastal, desert, forest, montane, wetland ecosystems and a host of others appear to be essential not only to understand the resource potential but also for a better idea of the different types of interaction among the biota.

4.8 Behaviour and survival of sympatric species in different ecological conditions: Species complexes are of common occurrence. Within a taxon, sibling species are known to exhibit biological differences with reference to their role in disease transmission, responses to insecticides, preferences to feed on the blood of man and their distribution in space and time.

The sibling species which are morphologically alike have specific ecological preferences thereby resulting in a specific distribution pattern in nature. Further, within a given ecosystem, they may have specific niche preferences with reference to breeding and resting. Knowledge of the environmental conditions which promote the existence and proliferation of such sibling species is essential and is particularly recommended for those taxa which are medically and economically important, in order to plan suitable control strategies.

The sibling species maintain a total reproductive isolation in nature even though they have been found to exist sympatrically. Further, in many extensively studied members of anopheline species, it has been well established that mechanical, seasonal and habitat isolation are not the reasons for their isolation suggesting that ethological barrier may be the major factor operating in mate recognition systems.

However, these mate recognition systems breakdown between some members of the group leading to the production of hybrids between the sibling species. In specific cases, this breakdown could lead to the mixing of genes responsible for disease transmission thereby converting a non vector eventually to a vector. In view of this, it is felt that studies should be undertaken to identify behaviour aspects responsible for their isolation and the environmental factors responsible for their breakdown.

5. NEW THRUST AREAS

Some of the important aspects of animal behaviour and ecology on which immediate research is required are:

5.1 Biological rhythms
5.2 Olfactory behaviour and pheromonal interactions
5.3 Sociobiology
5.4 Behaviour of polymorphic/sibling/sympatric species
5.5 Biological diversity
5.6 Population ecology and genetics
5.7 Succession and community dynamics
5.8 Animal–plant relationship including pollination biology and cecidology
5.9 Ecosystem energetics
5.10 Ecology of biological invasions
5.11 Predation and parasitism
5.12 Pedobiology
5.13 Evolutionary and adaptive strategies

6. HUMAN RESOURCES DEVELOPMENT

6.1 Summer/winter schools

To invite attention of senior as well as young scientists in India to take up research on animal behaviour, there is an urgent need of organizing summer/winter schools in specialized areas for a period of 2–3 weeks with a stress on methodologies which should be adopted for taking up research in the area of animal behaviour, ecology and evolution. To start with, experienced scientists may be identified to organize such summer/winter school in the general disciplines of

- Behavioural ecology
- Ethology and sociobiology
- Community ecology
- Vertebrate behaviour.

7. NATIONAL FACILITIES REQUIRED

7.1 Centres for behavioural Research

To minimise ad hocism in research, DST can identify 'DST Centres for Behavioural Research' in which ethological research can be supported on a long term or permanent basis with respect to staff and contingent grants.

7.2 Five-Year Programmes

To further intensify research within the thrust areas, it is suggested that certain themes should be selected for supporting projects during the next five years. These could be:

(a) Ecosystem/habitat based (ecology and behaviour of fauna of rainforest)

OR

(b) Region-based (ecology and behaviour of vanishing bird species in the Indian desert).

8. OTHER SUGGESTIONS

A fairly large number of projects (all of which do not fall strictly under purview of animal behaviour) have been financially supported by DST. As a consequence, field data and comparative studies have accumulated at an impressive rate. However a number of questions have been raised:

- Have we contributed to some new concepts/theories pertaining to animal behaviour, ecology and evolution?
- Have we developed new techniques?
- Have we created an impact at a national and international level?
- Have we trained enough young research workers to take up work on animal behaviour, ecology and evolution?

In my opinion, a spontaneous reply to all the above questions would be: not much.

Why not, what are the major constraints that these important disciplines of life sciences are not getting attention of the research workers in our country. Why are scientists not interested in carrying out work which can strictly be categorized as that on animal behaviour.

8.1 Project tenure

There are a number of practical difficulties with university/institute scientists to take up actual behavioural work whether it is to be carried out in laboratory, semi natural conditions or in the field. Besides teaching very little time is left with a university teacher to take up extensive field work. There is always a paucity of funds to establish enclosures for ethological work and there is no vehicular facility for undertaking a project the tenure of which is 3–4 years. In fact, it takes 10–15 months to get the staff appointed and for the purchase of equipments. By the time the real work starts, the project tenure comes to an end. Moreover, at present the state of affairs is that when after 3 years, the scientist submits another project on similar lines either to DST or to the Department of Environment or to UGC/ICMR, ICAR so that his work is continued on a permanent basis, more often, this effort does not succeed or there is a gap of one or two years between sanctioning of the two projects from the same source or from different agencies. As such, the research work continues of an adhoc basis or is aimed at getting PhD degrees to students. Consequently, no relevant continuation of the major theme is maintained. With a view to make the DST support to research more purposeful, the projects should be sanctioned for period of 5 years with a provision of extension of 2–3 years.

8.2 Project monitoring

Every project should be strongly monitored/evaluated every year by a senior scientist or an adviser on the report of whom funds for the next year should be released. There should be an in-built system within the project of providing funds for his/her travel etc.

8.3 Data bank

The annual final report of the projects financed by DST are usually lost as cyclostyled/mimeographed matters and the data contained therein are not usually available to other scientists. It is, therefore, suggested that:

(a) A data bank should be established either by DST or with national institute(s) where all the data emanating out of the DST-supported projects should be deposited. It should be a binding on the principal investigator to compile data in a set proforma for depositing it in this bank. A request can be made to a competent (or to a group of) bio-statistician(s) to generate such a proforma which may satiate needs of projects of varied nature.

(b) An executive summary of the final report, preferably incorporating basic data, should be submitted by the PI in a printed form. Funds for printing this abridged final report (about 20 pages) should be provided within the project. Printed copies could be sent to all conventional/agricultural universities/research institutes and to important libraries in the country and abroad.

8.4 Duplication of support

Some mechanism has to be developed so that the overlap/duplication of financial support by different agencies to the same/similar project to the same principal investigator or to his colleague in the same department could be minimized.

IMPORTANT AREAS OF RESEARCH

Vertebrates

1. Pheromonal interaction among fishes, amphibians and reptiles in sex recognition, migration, alarm responses
2. Chemical communication among mammals
3. Influence of habitat type on vertebrate mating systems
4. Behavioural changes in relation to population cycles in rodents
5. Evolutionary strategies in relation to mating in reptiles
6. Sociobiology of smaller carnivores, primates and their translocation.

Invertebrates

A. Behavioural ecology

1. Theoretical and field studies of social insects with a view to understanding the evolution of social behaviour

2. Pollination ecology
3. Use of behavioural data in developing potential biological control agents
    a. Work on predaceous coccinellids
    b. Investigations into behavioural studies on tachinids with a view to improve their effectiveness as biological control agents
    c. Study of biological factors which effect the success/failure of introduced insects as biological control agents
    d. Knowledge about the interaction between ants and parasites used as control agents
    e. Examination into the interactions between arachnids and insects
4. Behaviour of insect populations with special reference to their migration and diapause
5. Biological response of natural insects populations to pheromones with special understanding to the importance of extreme individuals
6. Qualitative and quantitative behavioural studies of symbolic association between insects and micro-organisms
7. Evolution of host selection behaviour during insect pest-host plant coevolution
8. Sensorial ultrastructure and behavioural diversity in insects
9. Behavioural diversity and food web components in aquatic ecosystem and energetics
10. Soil arthropods their behaviour, and their role as potential elements in trophic chains.

B. *Behavioural physiology*

1. Insects and behaviour-modifying chemicals
2. Feeding and nutrition in insects
3. Environmental and physiological factors regulating reproductive behaviour of insects
4. An examination into the biochemical changes in the internal milieu associated with feeding and mating drives in insects feeding on blood
5. Ovipositional site attractants of blood-feeding insects
6. Electrophysiological studies to identify receptor specificity and host selection behaviour in insects
7. Neuroendocrinological and exocrine studies of insects:
    a. Effect of hormones on pheromone perception and production in insects
    b. Role of hormones in accessory glands functions and their impact on insect reproduction
    c. Role of hormones in neural activities in insect
    d. Effect of insect hormones on biological activities of related animals like spiders, mites, etc.
    e. Effect of vertebrate hormones on insect life activities
    f. Endocrine rhythms in insects
    g. Importance of kairomones in insect behavioural studies, specially those of insect parasites
    h. Bacterial decomposition of organic matter that result in the production of kairomones leading to alterations in the characteristics of ovipositional sites.

Behavioural energetics

1. Estimation of energy budget of economically important insects, specially social and polymorphic forms and to explore the possibility of manipulation the mechanism(s) of energy allocation in these insects.
2. Energetics of vector insects.

Behavioural genetics

1. Genetics of behaviour especially on feeding rhythms of haematophagous insects.

MEDICAL BIOLOGY

1. Fast changing frontiers of bio-medicine have made tremendous impact on medical research. How to integrate these new developments in our research programmes is a major challenge for medical sciences in India, where major emphasis is still on data collection. While it is of utmost importance to generate reliable information on our disease pattern, simultaneously, it is imperative that modern developments in biology must be increasingly applied as investigative tools. Further, if we have to emerge as leaders, it is essential that we not only work in the front-line areas but our scientists must create new frontiers. This could only be achieved by promoting, in a major way, basic research which is the mother of all scientific discoveries.

2. Unfortunately, our medical research organizations lay very little emphasis on basic research. With the result that for generation of new knowledge, we still continue to depend heavily on the developed world. This is justified in certain quarters, on the

grounds that fundamental research is very costly and, therefore, with our limited resources, we cannot compete with the developed world. Often, the slogan 'no need to re-discover the wheel' is used to justify this approach.

3. As a consequence, the medical research has not made significant inroads either into the management of health affairs or in research in the academic sciences in this country.

4. This attitude, which is detrimental to research in medical biology, must change.

5. This can be corrected only by a well planned effort to promote fundamental research in medicine.

6. In a vast country like India, with enormous problems, it would be desirable to focus attention on a few priority areas to maximize utilization of limited resources.

7. The central theme of this approach paper is to identify the thrust areas in health sciences. Obviously, they would be determined by the prevailing disease pattern. Simultaneously, S & T planning should also be futuristic and, therefore, the changing health scene has to be taken into account. Diseases that are knocking at our doors should not be ignored.

8. Communicable diseases, that have vanished from the 'West', continue to be our dominant killers. They are responsible for high infant mortality, paediatric morbidity and economic loss in adults due to loss of wages. Strategies to control these disorders are by and large well known. Improved nutrition and personal and environmental hygiene hold the key to their control, which is now a more or less a managerial problem that needs little research inputs. However, it is recognized that there are lacunae in our knowledge about the host–parasite interaction and the factors that modulate immune response. A better understanding of the host–parasite interaction would provide clues to evolve strategies for immunodiagnosis as well as production of newer vaccines.

9. Despite tremendous progress in all sectors, continued unabated population growth has upset our economic growth. It is, therefore, felt that all novel approaches aimed at generating information on fertility regulation, and development of new contraceptives should be supported on priority basis.

10. Occurrence of cholera, almost in epidemic form in the capital, indicates the fragility of our health services. However, despite the shortcomings, life expectancy is doubled since independence. Therefore, in the next decade, age-related diseases such as cardio- and cerebro-vascular disorders, cancer and degenerative diseases will be of importance to us.

11. Thrust areas should also include technology-oriented topics such as computer modelling, patch clamp technique, etc.

12. Knowledge about the disease process is tremendously improved as soon as a laboratory model is established. All such efforts would be specially encouraged.

13. These have been the major considerations in suggesting the thrust areas (list enclosed). The list is not all inclusive and would be modified keeping in view the trends of discussions on this approach paper.

14. While recognizing the importance of collecting reliable data on disease pattern, this PAC would primarily like to support new, innovative idea-oriented interdisciplinary investigative projects aimed at understanding disease mechanisms.

15. Projects aimed at merely data collection, case reporting, drug testing, routine clinical and community field research would receive low priority.

16. One of the major challenges in medical biology is the *man-power development*. There are hardly any medical scientists involved in basic research. The main factors are the lack of motivation in most of the physicians, who prefer service and practice-oriented lucrative clinical work and have little aptitude for research. The medical curricula are somewhat outdated and hardly prepare students for a research career. The number of medical colleges have increased by 8 to 10-fold since independence. But in this process the quality has suffered. Majority of our medical colleges lack infrastructure required for basic research. Research is usually done through MD theses and lack depth and long-term perspective. Because of these factors the research culture is often lacking. As a consequence, much of our medical research still centres around mere data collection. Even in this area, unfortunately, little emphasis is paid to proper documentation, biostatistics and epidemiology. The results are not able to often stand the scientific scrutiny. The man-power developments in medical biology would need a change in the attitude and aptitude of our physicians. Teaching curricula, even at the high-school and pre-university levels, will have to be up-dated to include developments in contemporary sciences. Simultaneously, the medical curricula need drastic alterations to train our physicians more as scientists and not only as 'healers'. This is obviously a long-term complex issue.

17. Much could be achieved, even in the prevailing conditions, by promoting collaborative research between medical scientists and basic biologists. Physicians often find themselves somewhat deficient in knowledge about modern biology and, therefore, feel shy of undertaking investigations using newer emerging technologies. On the other hand, the basic scientists, who are equipped with such knowledge, have little appreciation of disease process. As a short-term measure, this imbalance could be removed, to some extent, by supporting workshops, seminars, training programmes, etc. of interdisciplinary nature on disease-oriented modern biology. Topics for such activities would be identified through broad-based consultation with members of different disciplines.

18. Infrastructural facilities: Majority of our medical colleges, and even the universities, are poorly equipped to conduct front-line bio-medical research. To support investigative laboratory-based research, it would be essential to strengthen infrastructural facilities, specially in newly emerging areas, so that the investigators are equipped to use modern technology. As the first step, this PAC recommends that modest infrastructural facilities could be developed in a few medical colleges in different parts of India, specially in the university towns, so that common forum is provided for physicians and biologists to work together.

19. International interaction specially with neighbouring nations: This PAC would like to enlarge the scope of its activities and promote collaborative research, specially with the neighbouring SAARC countries. As the first step, the PAC would like to organize small interdisciplinary group meetings, seminars and training programmes on common bio-medical problems. It is hoped that such activities would provide meeting ground for bio-medical scientists of the neighbouring countries and enable them to identify and organize collaborative research projects. Such workshops could be held under bilateral agreement involving, if necessary, international agencies. For this purpose, the PAC would like to identify a few leading bio-medical institutes which could provide opportunities and facilities for guest scientists from the neighbouring countries.

LIST OF THE SUGGESTED THRUST AREAS

1. Cellular and molecular aspects of host-parasite interaction including the study of factors modulating immune response in relation to our dominant communicable disorders
2. Novel approaches to the development of vaccines specially polyvalent vaccines relevant to India
3. Fertility regulation
4. Infant and childhood diarrhoeas, their etiology and mechanisms, specially the aspects of gut immunity
5. Molecular biology of genetic disorders
6. Thrombosis and platelet biology, specially in relation to cardio- and cerebro-vascular accidents
7. Cellular and molecular aspects of cell transformation, growth signalling, metastasis and cancer chemotherapy
8. Epidemiological modelling
9. Computer modelling
10. Whole body energetics
11. Patch-clamp techniques
12. Material science, specially the study of novel biopolymers/bioimplants
13. Animal models in human diseases.

IMMUNOLOGY

1. PREAMBLE

The Science and Engineering Research Council of the Department of Science and Technology, Govt. of India has adopted an approach of supporting creative research in a major way in a few selected areas of research identified as Thrust Areas for the period 1980–1990. The objective was to concentrate effort in newly emerging and frontier areas of interdisciplinary fields to make significant impact on the scientific scene. The time has come to review the progress made and have a look for future directions.

In the field of medical sciences (which was one of the four areas under life sciences) immunology was given the pride of place. Immunological control of tropical diseases (in early part human reproduction was also included) was identified as one of the two thrust areas under medical sciences. However, there have been few takers of this opportunity. Whatever proposals came were predominantly in the area of immunodiagnostics and monoclonals. Some other proposals focused on the evaluation of immune response and understanding of pathogenesis. It is only in recent years that some basic and frontline proposals have started coming.

Immunology continues to play a dominant role in modern medical research. Understanding the pathogenetic process of immune-mediated disorders on one hand and tailoring of vaccines as well as successful modulation of immune response on the other hold promise of providing solutions to control of more difficult to manage infectious diseases as well as malignancies. Monoclonals, lymphokines, interferons and other molecules which can act as biological response modifiers are on centre stage. Equally importantly the discipline of clinical immunology dealing with diseases of immune organ is gaining ground. Emergence of AIDS has not only forced to take immune deficiency seriously, but has also given a major boost to study the immune system especially in the western world. The progress in the field of immunoinflammatory diseases is also spectacular. Emphasis has shifted from symptomatic anti-inflammatory agents to disease-modifying drugs/immunomodulating agents. However, inspite of some success the very basis of autoimmune diseases continues to remain an enigma. Considerable advances have been made in understanding the structure and function of HLA molecules and their disease association but the role of genetic regulation of immune response in humans still remains hypothetical. Cell sorting and cell cloning could provide better understanding of specific subsets of cells but without understanding of idiotype, clonotype and cellular networking in the immune system, our understanding of aberrations of immune response and their control will remain a challenge. This is also applicable to genetic engineering of vaccines and molecules of biological response modifiers which have far reaching potentials.

In the above context there could be little doubt that research in the field of immunology continues to remain a thrust area. However, there is great need to precisely delineate specific areas in this field where Indian scientists could make meaningful contribution and international impact. Equally important are the aspects of research and development in the field of immunology which will help us deliver better health care to the population of India. There is also a need to strengthen manpower base and develop adequate infrastructure facilities which can support research in frontline areas.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

Infectious diseases continue to remain the major cause of morbidity and mortality in India. Besides, other diseases or situations where knowledge of immunology could be applied (except for AIDS) appear to be no different in our country from those in the West. With the population base of our country, the absolute dimensions of these problems could be huge in India. In a recent population survey it was found that only one of the immunoinflammatory diseases, viz. rheumatoid arthritis had a point prevalence of 1.18%, giving a projected prevalence of over 7 million patients in India.

In India, there are about 40 centres and over 400 immunologists who are engaged in research in immunology (Appendix 1). If the number of papers presented at the annual meeting of the Indian Immunology Society is taken as an indicator there has been modest but continuous increase in research activity in this field (125, 152 and 206 abstracts were submitted in 1985, 1986 and 1987, respectively). A full fledged National Institute of Immunology has been established at New Delhi. In addition the facilities for immunological research have been either created or strengthened in many universities, national institutes and medical institutions. More than a dozen laboratories have been reported to be engaged in raising monoclonals in various parts of the country (Appendix 2). Research in this field has been actively supported by DST, ICMR and CSIR. To monitor the menace of AIDS, ICMR has established a network of screening centres. Lately research and developmental effort in the field of immunology has also been picked up by the industry. One of the most spectacular of these is the high technology laboratories of ASTRA at Bangalore. Immunodiagnostics and Hoechst (Boehringer) have marketed several immunological reagents and test kits in India. In particular, the immunological tests for pregnancy are now marketed by several firms. Ranbaxy has also entered in the field of immunologicals. Several others are promoting the reagents of the foreign principals. However, the supply of these reagents is still not satisfactory. One of the major limiting factors for the progress of laboratory investigations in this field has been lack of availability of tissue culture grade plasticware in India.

During the period from 1979 to 1986 DST has funded 19 research projects in the field of immunology for a total cost of 161.95 lakhs (Appendix 3). This accounted for 22% of the projects (as well as funds) sanctioned in the field of medical biology as a whole. More than 100 publications have emerged from these studies and 26 persons have qualified for PhD. Besides, at least 100 technical persons have been engaged in these research projects. Out of 19 projects 11 were in the field of infectious diseases and 5 on cancer.

ICMR has supported a large multicentric study on seroepidemiology of malaria and another one on HLA typing. Besides, its own institutes have also been conducting research on various aspects of immunology. The same has been true of CSIR laboratories as well.

An analysis of abstracts presented at the Indian Immunology Society indicates that the largest number (172) of papers dealt with infectious diseases. It was followed by studies on malignancies (73), clinical aspects (57), basic aspects (53), reproductive immunology (28), immunogenetics (20), immunopharmacology (19), veterinary immunology (6) and allergy (5). It should only be taken as representative of trend only because papers dealing with immunological aspects are presented at several other forums as well (Indian Association of Medical Microbiology, Indian Rheumatism Association, etc.).

On overall analysis, Indian immunologists have not yet graduated into modern immunology. Barring aside few laboratories the activity has mainly revolved around development of immunoassays and evaluation of immune competence (quantitation of immunoglobulins, subsets of lymphocytes, etc.). Hybridoma technology and genetic engineering for vaccine development are still in infancy. Of late there are some signs of recovery but a very intensive effort is required to join the mainstream. A few high level workshops have been held in recent years to develop the manpower but there is no follow-up information on the outcome of these exercises.

3. INTERNATIONAL STATUS

The field of immunology is one of the most active fields of research internationally. The number of Nobel awards, books and journals, national and international conferences in the discipline and the volume of marketed immunologicals and immunochemicals is a good testimony to it. The great advances in immunology in recent years have mainly resulted from influx of investigators from multiple disciplines ranging from highly molecular to highly clinical areas. There is no area in biology that has remained untouched by immunology and there is no methodology that has either not given or accepted something from it. In recent years, more and more emphasis is being given to understand the structure and function at molecular level. Some of the areas that are being hotly pursued include T- and B-cell biology, T-cell receptors, anti-idiotypes, lymphokines, monokines and their receptors, antigen recognition and processing at molecular level, antibody combining site, regulation of antibody class expression, molecular mechanisms of signal transduction in immunocytes, non specific MHC-unrestricted killer cells and their receptors, regulatory structures on the V and C regions of immunoglobulin molecules, immune-response gene function, genetic aspects of antibody V regions, genetic origin of auto-antibodies and origin and significance of anti-DNA antibodies, etc. In the area of immune intervention in disease states significant progress has been made in the form of development of new generation vaccines, monoclonal antibodies as therapeutic measures, anti-idiotypic antibodies as vaccines, interleukins, interferons, certain compounds identified as immunomodulators and total lymphoid irradiation. Significant strides have also been made in the field of transplantation immunity including cardiac, bone marrow, renal and other organs transplantation. The modern pandemic of AIDS is also being chased from various angles including the prospects of development of potent antiviral drugs, immune response modifiers and vaccine. It is humanly impossible to review the entire field in this space.

4. GAP AREAS

5. NEW THRUST AREAS

There is so much going on and at such high level in the field of immunology in the rest of the world that it would be impossible for us to match unless efforts are intensified and concentrated towards areas of national relevance. This requires taking concious decisions in identification of areas for vigorous pursuit. This approach does not preclude independent research.

In identifying thrust areas the debate about basic and applied is futile. The choice has to depend on available resources, both in manpower and material. The ideal would be that all the active labs are visited and discussions are held with the scientists in these labs about their interests and future plans. The identified thrust areas should match our capabilities. However, since the available base is limited we will also have to identify areas which are contemporary and befitting with national needs to which scientists can be attracted. In the latter category the following areas deserve consideration:

5.1 Immunochemical characterization of Indian strains of parasites and other infectious agents; identification of their protective antigens and functional epitopes and understanding of factors determining immune response (antigen recognition, presentation, processing, cell interactions, effect molecules and cells, etc.) to these epitopes, including genetic factors (HLA, IR genes and T-cell receptors) and idiotypic net working mechanisms.

5.2 Development of monoclonals and immunodiagnostics.

5.3 Identification of genetically determined variations in immune response including genetic immunedeficiency diseases and aberrant immune responses including cancer immunology and that of immunoinflammatory auto-immune diseases for understanding the development and functioning of immune apparatus by dissecting the underlying mechanisms of variation.

5.4 Search for the immune modulators and other biological response modifiers among indigenous systems of medicine including clinical evaluation of emerging agents.

5.5 Development of genetically engineered vaccines of national interest.

6. HUMAN RESOURCES DEVELOPMENT

More important than financial constraint is the constraint of available trained manpower in the field. Immunology is not yet commonly adopted as a standard undergraduate or postgraduate subject. It is mostly taught as a part of zoology, biology, or life-sciences and that too only at few places. Its content in medical school curriculum is negligible. The only source of manpower in the field of immunology is PhD programmes. The latter are essentially project-based and do not include broad-based formal training. There is an urgent need to generate human resource in immunology if we wish to make meaningful contributions in this field. The following is suggested:

6.1 Courses in immunology with laboratory facilities should be included in graduate, postgraduate and medical curricula all over the country. Optional specialization in immunology can be offered at postgraduate level.

6.2 A regular summer/winter school in immunology be held at an identified place every year on a long term basis.

6.3 More frequent intensive workshops to be held in different parts of the country according to expertize of individual laboratories which should include a follow-up programme as well.

7. NATIONAL FACILITIES REQUIRED

Research has become highly technical these days. Some of the requirements are quite fastidious and often have to be imported. High-tech research is infeasible without such facilities. Unfortunately, the industry does not seem to be ready to accept this challenge. The only recourse is to establish national facilities. Some of the requirements for research in immunology are identified as follows:

7.1 SPF defined strains of inbred mice

7.2 Bank of Indian strains of parasites and other infectious agents

7.3 Cell banks of immunological clones including target cells, lymphokine-producing cells and hybridoma clones secreting commonly used phenotype and marker-specific monoclonals

7.4 Reference antigen and sera banks providing international and national standard of antigens, and positive and negative antisera, conjugated second antibodies, etc.

7.5 Facilities for setting up hybridomas.

Simultaneously, it is also important to take steps for

— developing our own clones producing specific reagents and,

— improving the supplies of immunological reagents and tissue culture grade plasticware. Although it is not in the realm of research but it calls for strong administrative action.

APPENDIX 1

Centres engaged in research in immunology

1. All India Institute of Medical Sciences, New Delhi
2. Aligarh Muslim University, Aligarh
3. Amla Cancer Research Centre, Trichur
4. Banaras Hindu University, Varanasi
5. Biology Group and Radiation Medicine Centre, Bhabha Atomic Research Centre, Bombay
6. Cancer Research Institute, Bombay

7. Cancer Research Institute, Madras
8. Central Drug Research Institute, Lucknow
9. Central Jalma Institute of Leprosy, Agra
10. Centre for Biochemicals, Delhi
11. Centre for Cellular and Molecular Biology, Hyderabad
12. Chittaranjan National Cancer Research Centre, Calcutta
13. Christian Medical College, Vellore
14. Hindustan Ciba-Geigy Research Institute, Bombay
15. Cytology Research Centre, New Delhi
16. Foundation for Medical Research, Bombay
17. Haffkine Institute, Bombay
18. Haryana Agricultural University, Hisar
19. Institute for Research in Reproduction, Bombay
20. Indian Institute of Science, Bangalore
21. Institute of Immunohaematology, Bombay
22. Institute of Pathology, New Delhi
23. Industrial Toxicology Research Centre, Lucknow
24. Indian Veterinary Research Institute, Izatnagar
25. Jawaharlal Nehru University, Delhi
26.. K.E.M. Hospital, Bombay
27. K.G. Medical College, Lucknow
28. Madurai Kamaraj University, Madurai
29. Malaria Research Centre, New Delhi
30. National Institute of Communicable Diseases, Delhi
31. North Bengal University, Siliguri
32. National Institute of Immunology, New Delhi
33. National Institute of Virology, Pune
34. National Institute of Nutrition, Hyderabad
35. Post-Graduate Institute of Medical Education and Research, Chandigarh
36. Regional Cancer Research Centre, Trivandrum
37. Regional Medical Research Centre, Bhubaneswar
38. Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow
39. Sher-i-Kashmir Institute of Medical Sciences, Srinagar
40. Tuberculosis Research Centre, Madras.

APPENDIX 2

Laboratories involved in raising monoclonal antibodies in India

S. No.	Institution	Monoclonal antibodies against
1.	National Institute of Immunology, New Delhi	(i) *E. histolytica* (ii) HBsAG (iii) *S. typhi* (iv) *M. tuberculosis*
2.	All India Institute of Medical Sciences, New Delhi	(i) Herpes simplex virus types 1 and 2 (ii) *S. typhi*
3.	Cancer Research Institute, Bombay	(i) Myeloid leukemic cells (ii) Alphafetoprotein (iii) Squamous cell carcinoma, oral cavity
4.	Tata Memorial Hospital, Bombay	Myeloid leukemic cells
5.	Foundation for Medical Research, Bombay	*M. leprae*
6.	Institute for Research in Reproduction, Bombay	Thyroglobulin
7.	Hindustan Ciba-Geigy Research Institute, Bombay	Filaria
8.	Cancer Research Institute, Madras	Lymphoma-specific antigen
9.	National Institute of Virology, Pune	*Japanese encephalitis* virus
10.	School of Biological Sciences, Madurai Kamaraj University, Madurai	Porin (*S. typhi*)

APPENDIX 3

DST-funded research projects in the field of immunology

S. No.	Title	Principal investigator	Institution	Period	Grant (Rs. in lakhs)
1.	Etiological factors, pathogenic mechanism, immunological status and appropriate dietary schedule in intractable diarrhoea patients	O. P. Ghai	AIIMS, New Delhi	84–87	9.14
2.	Immunodiagnostic and other studies in Bancroftian filariasis	B. C. Harinath	Wardha	79–82	9.47
3.	Immunity in amoebiasis: study of the host–parasite interaction at the macromolecular level	Sohail Ahmed	AMU, Aligarh	80–83	4.01
4.	Evaluation of endogenous interferon inducers and antiviral drugs for the prophylactic and therapeutic use in man	S. N. Ghosh	NIV, Pune	80–84	4.43
5.	Immunological studies in typhoid fever	Ramesh Kumar AN Malviya	AIIMS, New Delhi	81–83	—
6.	Cell-mediated immune responses in childhood tuberculosis	V. Seth	AIIMS, New Delhi	81–85	—
7.	Hybridoma produced monoclonal radiolabelled antibody in specific targetting both in neoplastic and infective lesions	D. K. Hazra	S. N. Med. College, Agra	84–88	—
8.	Detection of antigens for a rapid and improved laboratory diagnosis of typhoid fever	Ramesh Kumar	AIIMS, New Delhi	84	6.71
9.	Interaction between nutrition, immunology and drug response in man	N. A. Kshirsagar	GSMC, Bombay	82–86	4.0
10.	Use of monoclonal antibodies in the studies of development and differentiation of nervous system TSA	Shail Sharma	AIIMS, New Delhi	82–86	4.0
11.	Epidemiological study of viral hepatitis and immunological aspects in pregnancy	R. S. Karrat	Haffkine Inst., Bombay	83–87	1.0
12.	Studies on the characterization and protective roles of defined antigen of axenic *E. histolytica* by use of monoclonal antibody	V. K. Vinayak	PGIMER, Chandigarh	86–89	
13.	Lepromin conversion, natural or induced by agents, and its competency in clearing *M. leprae*	S. Chaudhary	School of Trop. Med., Calcutta	85–86	5.0
14.	Role of IL-1 in microbicidal killing in pulmonary TB	R. S. Padma	AIIMS, New Delhi	86–88	1.0
15.	Assessment of immunological alterations caused by some common pesticides in experimental animals	B. D. Banerjee	NICD, Delhi	85–87	0.9

16.	Studies on the HLA system with special reference to distribution, genetics and disease association	R. M. Pitchappan	MKU, Madurai	84–88	10.6
17.	Immunodiagnostic tests for detection of asymptomatic malignant neoplasms	Uday Chander	CRI, Madras	81–85	29.45
18.	Development of monoclonal antibodies reactive against tumour or associated antigens of human leukemias and murine mammary tumour virus	Sudha Gangal	CRI, Bombay	84–87	7.62
19.	Monoclonal antibodies to lymphoma associated antigen (LAA) and their application in early diagnosis and therapy	Uday Chander	CRI, Madras	85–88	13.43

NEUROBIOLOGY AND MECHANISMS OF BEHAVIOUR

1. PREAMBLE

FOLLOWING the 'Baroda Seminar', SERC identified thrust areas for major research programmes for 1980–90 period. Among the areas identified in the life sciences there were two primarily concerned with the broad field of neuroscience—Neurobiology and mechanism of behaviour, and Human neurobiology in relation to mental health.

Realising the overlap between these two fields a single PAC was constituted to encourage work in these areas. The paucity of ongoing research in these areas became very apparent during the initial meetings of the first PAC on neurobiology. It was, therefore, decided to organise a 'Brain Storming Session', to take stock of the existing situation and stimulate future activity. This session was held at Bangalore in November 1984. The proceedings and recommendations of this session were widely circulated. This resulted in a distinct increase in interest in the field, though much more needs to be done. The present report provides a review of the current status and suggestions for future directions. This is based on discussions in the PAC on neurobiology on several occasions, as well as comments received from some other colleagues working in this area who gave their suggestions on the basis of a preliminary paper circulated to them.

2. STATE-OF-THE-ART IN NATIONAL CONTEXT

A survey of neurosciences activity in the country reveals that in various fields of neurosciences, neuroanatomy, neurophysiology, neuropharmacology and toxicology, neurogenetics, neuropathology, clinical neurological and behavioural sciences, nuclei and groups exist scattered all over the country. There was unmistakable evidence of some very good and a few outstanding units in each field, but in general the overall activity in this very important field is still limited. There is a real dirth of interdisciplinary, comprehensive and coordinated activity cutting across the artificial barriers. As a matter of fact most workers belonging to any of the disciplines mentioned above, not only seldom interact with each other, but are not even fully aware of the existing expertize available. Neuroscience research is a continuum of study from the molecular to the behavioural level. To quote the office of Science and Technology Policy, USA (1985): Neuroscience was defined as 'that body of research directed towards understanding the molecular, cellular, intercellular, processes in the central nervous system and the ways in which those processes are integrated in CNS functional control system, with emphasis on research relating CNS functions with behaviour.

The Thrust Area Programmes (1980–90) identified the following subjects in the field of neurobiology.

Neurobiology and mechanisms of behaviour.
Biophysics of the nervous system
Sensory and motor mechanisms
Brain structure and development
Higher functions of central nervous system (learning memory)

Chemical modulation of CNS functions

Neurogenetics

Human neurobiology in relation to mental health.

Neurobiological investigations with emphasis on neuronal transmission mechanisms, psychopharmacology developmental neurobiology of human foetus.

As a result of the efforts of the PAC during the last 5 years SERC supported the following programmes:

2.1 Units supported under the IRHPA programme

Unit of Neurobiology and Mechanism of Behaviour at the School of Biological Sciences, MKU, Madurai. (Programme Director, Prof. M. K. Chandrashekaran 1983–88): This unit has done creditable work on the nature of entrainment of circadian rhythms, social synchronization of these rhythms, electrophysiology of ecolocation in bats. Besides, a chronocubicle complex with isolation facility for experiments with human subjects, the only of its kind in the country and one of the few in the world, has been established and is currently being regularly utilized.

Unit on Neural Transplantation in Mammals at AIIMS, New Delhi (Programme Director, Prof. P. N. Tandon): A centralized facility to initiate research and training in this frontier area was established in March 1986. This unit has already carried out a variety of embryonic neural transplants in adult rodents and monkeys. It has organized two National Training Programmes.

2.2 Individual projects

During the last five years the following individual projects were supported and a few others are now being evaluated.

- Studies on myelin synthesis in developing brain—Prof. P. S. Sastry, IISc., Bangalore.
- A comparative study of nervous system and platelets with particular reference to the amine-sensitive arylacylamidases and acetylation – deacetyltion processes - Prof. A. S. Balasubramanian, CMC, Vellore.
- Functional organization of the skeleto-motor correlates of the hand with respect to skilled movements—Prof. M. S. Devanandan, CMC, Vellore.
- Identification of domains responsible for arylacylamidase and peptidase activities in acetylcholinesterase and pseudocholinesterase—Prof. A. S. Balasubramanian, CMC, Vellore.
- Mechanisms of peripheral nerve damage in leprosy and approaches for its prevention and treatment—Dr. N. H. Antia, The Foundation for Medical Research, Bombay.
- Studies on neurotransmitter receptors in platelets of Parkinsonian patients—Prof. Devika Nag, KG Medical College, Lucknow.

2.3 Training programmes

- National workshop on reconstruction of the central nervous system: Organised by Prof. P. N. Tandon, AIIMS, New Delhi, 1986.
- National course in basic neurosciences for postgraduates in clinical neurosciences: Organized by Prof. P. N. Tandon, AIIMS, New Delhi, October 1987. Second course planned from November 14 to 25, 1988.

Obviously this is not the only activity going on in the field of neuroscience in the country. Unfortunately there is no single source from where one could gather a comprehensive information on the subject. The need for creating a 'National Neuroscience Information Unit' has already been approved in principle by SERC.

2.4 Work going on in the country in the areas identified by SERC though not necessarily supported by DST is given below. This information is based on the personal knowledge of the members of PAC and hence is most likely incomplete.

Neurobiology and mechanism of behaviour

Besides the work at the Madurai Unit mentioned above the Department of Physiology of the AIIMS has a continuing interest in this field over the last two decades. The role of limbic system, hypothalamus and other regions of the brain in a variety of behaviours, e.g. feeding, sex, reproduction, sleep and response to pain have been the subject of a large number of studies.

– *Biophysics of the nervous system*

We are not aware of any significant contributions in this field from any individual or group in the country.

– *Sensory and motor mechanisms*

Some work is going on in this area at CMC, Vellore; AIIMS, New Delhi, and NIMHANS, Bangalore.

– *Brain structure and development*

The group at AIIMS is undoubtedly the most active group in this field. Their work in the field of development of the visual and pain pathways in human embryos is certainly of international standard. Important contributions have also been made by this group on the effect of malnutrition, radiation and thyroid hormone on developing brain.

Three or four groups, i.e. at AIIMS, PGI, NIMHANS, Institute of Neurology, Madras and Bombay are actively involved in the field of neurooncology. More basic investigations are, however, necessary to improve our understanding of the brain tumours, a majority of them still not being curable.

– *Higher functions of central nervous system (learning, memory)*

To the best of our knowledge hardly any activity is going on in the country in this vital area. The studies on neurophysiology of Yoga carried out initially at AIIMS, New Delhi and currently at NIMHANS, Bangalore, are the only important contributions worth mentioning.

– *Chemical modulation of CNS function*

A few groups at Vellore, Bangalore, New Delhi, Lucknow, Hyderabad and Calcutta are working in the field of neurochemistry and chemical modulation of the central nervous activity.

– *Neurogenetics*

The group at TIFR Bombay, has made outstanding contributions in this field. Some work has also started in CCMB, Hyderabad, on molecular genetics, which has bearing on neurogenetics. Department of Zoology, BHU, Varanasi has been working on genetics of aging which has some relevance for CNS.

It would, thus be seen from the above account that the overall activity in the field of basic neuroscience in India is grossly inadequate. There are very few individuals/groups/institutions currently engaged in this field. The importance of this field can hardly be overstated. Better understanding of the human brain and behaviour may be the key to survival of the human race itself. Even in the short term, a large number of neurological and psychiatric disorders, till now ill understood and thus untreatable, demand urgent attention for better understanding and relief.

3. INTERNATIONAL STATUS

The extensive excitement witnessed globally in the field of neuroscience is reflected in the increasing number of scientists engaged in research in this area all over the world. In USA in 1971, the Neuroscience Society had 250 members, which in 1985 had increased to over 8,000. In China in a single institute in Beijing or Shanghai there were more scientists working in narrow areas like visual or auditory system than the total number of neuroscientists in India. This spurt of interest in the field is because of the recent developments in diverse fields like molecular biology, biotechnology, imaging and biochemical techniques, advances in solid state electronics and computers. Thus, new analytical techniques like mass spectroscopy, nuclear magnetic resonance spectroscopy, high pressure liquid chromatography, radioimmunoassay, etc. now permit isolation and identification of specific molecules used by nerve cells for intercellular communication. It is now possible to identify and characterize the gene sequences responsible for the various neuropeptides, neuromodulators and neurohormones and thus modify any defects. The technique of 'patch clamping' enables an investigator to record from the tiniest of neurons and detect the opening and closing of a single ion channel as a small patch of membrane responds to neurotransmitters. Much useful information on the cellular properties of the nervous system is being gained through the use of cell-culture, *in vitro* study of brain slices and more recently using neural transplantation. The technique of genetic hybridization today permits development of animal strains with specific neurological or behavioural properties, ranging from resistance to alcohol intoxication to the ability to acquire new reflex skills. These strains then permit studies for their underlying structural or biochemical defects.

The techniques for recording the activity of the brain during behaviour have expanded greatly. Methods now range from study of the activity of single neuron in animals to the ability to visualize regional cerebral blood flow or metabolism in intact human brain. Thus nuclear magnetic resonance, imaging and spectroscopy, positron emission tomography, single photon emission computerized tomography, telemetry, electromagnetic field registration, etc. have lead to sophisticated analysis of behaviour hitherto impossible to imagine.

Intense activity is going on in the field of artificial intelligence, computer modelling and simulations, to provide better understanding of neural activity on the one hand and simulate it for development of more sophisticated and powerful computers on the other.

These techniques have thus made it possible to explore certain basic questions about the brain, its growth, connectivity, organization, means of intercellular communication, anatomical and biochemical circuitory which have direct relevance to the understanding of its functions in health and disease. Neural transplantation, with its vast potentialities for relief of neurological disorders, like Parkinsonism, dementia, etc. has already attracted thousands of neuroscientists around the world. It is already a reality, its wider application depends upon a better understanding of the underlying biology.

Global reviews further highlighted that today for any worthwhile neuroscience research the traditional compartmentalized efforts are not likely to succeed. Modern techniques of molecular biology, cell culture hybridoma technology and immunology are equally important for workers in most areas of neuroscience referred to above. Furthermore it is obvious that to break new grounds inter-disciplinary interaction is necessary not only among the scientists in various neuroscience disciplines but also with mathematicians, physicists, chemists and computer scientists. It is neither necessary nor desirable to catalogue all possible areas of research in neuroscience. However, on the basis of the discussion some general guidelines emerged on identification of new thrust area, which are listed here not necessarily in their order of priority or importance. Nevertheless this list is drawn taking into consideration the existing talent, potentials and the national needs.

4. GAP AREAS

From the above description it will be seen that there are major areas of research in neuroscience which are either being pursued at subcritical level or not at all, cataloguing these would serve no useful purpose. However, the areas identified for preferential support are given under the next section.

5. NEW THRUST AREAS

5.1 *Developmental neurobiology*

The large resource of human foetuses and adult brains from autopsies available in the country could be exploited to study developmental neurobiology utilizing modern neuroanatomical, and neurochemical techniques combining various newer labelling methods (antegrade and retrograde Golgi, double labelling, immunoflourescent and immunocytological investigations) along with quantitative morphometery routine, flourescent and electron-microscopy, on the one hand and evaluation of regional distribution of various neurotransmitters and neuropeptides on the other.

This would provide valuable information on (a) the development of normal human brain, its organization, biochemical correlates and connectivity, since large areas of human brain even today remain uncharted, and modern techniques can provide quick and reliable information which was not possible till recently, (b) this basic information about the normal state is important to answer a large number of questions regarding pathological states.

– *The effects of malnutrition*

A large amount of controversial data exist on this subject, however, specific answers are not yet available for many vital questions of great practical importance. Thus so far quantification of the damage, its precise extent, pathogenetic mechanism, and reversibility, etc. have not been possible because the available techniques till recently were inadequate or too slow or too complicated to answer these questions.

– This knowledge could be utilized to understand the basis of a number of ill-understood developmental defects, and permit quantitative study of changes associated with ageing.

– It is likely to throw new light on plasticity and regeneration of the nervous tissue both at the central and peripheral levels. This information is of great practical value in managing patients with neurological damage. To enable such studies to be carried out it will be necessary to develop facilities for:

- Foetal and adult brain banks
- Quantitative morphometry
- Modern labelling techniques, specially possibilities to raise monoclonal antibodies and various marker isotopes
- Microanalytic methods to measure minute levels of neurotransmitters/hormones/modulators, etc.
- Tissue culture, and explant and organ culture,

which can help provide several answers in neurobiology

- High-voltage electron microscope facilites.

Besides enhancing our knowledge of basic neurobiology such studies are directly relevant for the newly emerging area of neural transplant for treatment of human diseases.

5.2 *Neurophysiology*

A number of new techniques for neurophysiological studies have developed in recent years. Thus, in addition to the classical macro- and micro-electrode studies, patch-clamp technique, studies of brain slices *in vitro*, quantitation of behavioural parameters using computerized facilities and telemetry are some of the examples, which need to be developed.

5.3 *Neurochemistry*

Recent developments in neurochemistry have opened up completely new vistas to study normal and abnormal nervous system from the molecular to behavioural level. The existing expertize in this field could be utilized and strengthened to permit research to the frontier areas, static and dynamic studies of conformation of the transmitters and receptors and their modification by drugs and pathological states.

5.4 *Neuropharmacology*

Pharmacodynamic and pharmacokinetic action of known drugs, development of newer drugs utilizing computer modelling techniques are two emerging areas. Investigations for developing reliable and cheaper monitoring kits for drug levels, for clinical use need to be encouraged, studies on selected drugs used in traditional systems of medicine for modifying CNS function, need to be explored.

5.5 *Neuroimmunology*

A number of neurological disorders are now being recognized to have an immunological basis. On the other hand involvement of nervous system in regulation of immune system is no more a matter of speculation. In view of the available evidence of the role of CNS in modulating the immune system, studies are needed both to delineate the role of central mechanisms in immune diseases and to evaluate the usefulness of centrally acting drugs to modify the same.

5.6 *Neurogenetics*

It is obvious that the techniques of modern genetics could be used not only to study the manifestations of various genetic traits for normal and abnormal structure and function of animals (and human), but these new techniques could be utilized to understand some of the basic neurobiological mechanisms/phenomenon, e.g. neuronal organization, connectivity and even, behaviour.

5.7 *Neuroendocrinology*

This is a fast developing field still in its infancy in India. Facilities for modern neuroendocrinological investigations are available in only few centres. These studies are of great importance to understand normal growth and development, effect of undernutrition and malnutrition on mental development (classical example iodine deficiency and mental retardation).

5.8 *Study of pathological states*

Whole range of pathological states affecting the nervous system, central and peripheral, remain ill understood or un-understood. Some of these disorders specific to India are of greater interest to us. These are malnutrition, infective disorders (tuberculosis, leprosy, viral and parasitic), degenerative disorders (motor neuron disease, ataxias), and certain toxic substances of national relevance (lathyrism, connibas, country liquor).

Another important area is that of neurooncology, with the availability of newer techniques like *in vitro* and *in vivo* cell kinetics tissue culture, use of tumour markers, etc. It has now become possible to investigate brain tumour to get a better insight in their histogenesis, biological behaviour and response to chemotherapy, etc.

5.9 *Neurotoxicology*

Neurotoxicology is of great concern in view of the increasing exposure of man to a variety of neurotoxins of pharmacological, environmental and industrial origin. Furthermore, recent studies have provided a clue to the role of neurotoxins in the aetiopathogenesis of several neurodegenerative disorders such as Parkinsonism, motor neuron disease, dementia, etc. The following areas of research are, therefore, identified.

5.9.1 *Development of animal models*

Animal model need to be developed for testing of environmental toxins and industrial pollutants; priority should be given to solvents (particularly hydrocarbons and halogenated hydrocarbons), heavy metals (lead and manganese), pesticides and polymers.

An integrated approach using biochemical, electrophysiological, behavioural and morphological methods has to be used for the development of animal models. Biochemical evaluation would essentially involve the study of receptor changes, glycolytic enzymes and marker enzymes for subcellular components. Electrophysiological studies of peripheral and central nervous system including electromyography, neuro-muscular transmission, nerve conduction and evoked potentials are necessary to provide essential information of the functional status. Behavioural studies would involve motility monitoring, maze test for memory and tailflick test for pain sensation. Morphological studies of peripheral and central nervous system using histological, histochemical and ultrastructural methods, need to be carried out.

5.9.2 *Study of mechanism of action of neurotoxins*

It is important to delineate the precise site of action of neurotoxins. To achieve this objective, *in vitro* methods using brain slices, tissues cultured cells and subcellular fractions have to be developed. These studies would be critical in devising, *in vitro* assays for rapid screening of potential neurotoxins, while *in vitro* testing cannot completely replace *in vivo* studies, such methods would obviously reduce the number of compounds that need to be tested *in vivo*, saving valuable animals, time and expense.

5.9.3 *Application to human studies*

The information obtained from basic work has to be applied to the actual human situation. Well designed neuroepidemiological studies will provide the framework for obtaining prevalence data and allow intervention methods. Such studies will have to be conducted in selected population at high risk of exposure.

- The neurotoxicological data obtained from animal studies will be valuable in determining the maximum permissible levels of exposure. In the Indian context, the influence of nutritional status has to be given due consideration while formulating the guidelines. Therefore, studies have to be designed to assess the inter-relationship between the effect of toxin and nutritional factors.

5.10 *Biological psychiatry*

Another area of behavioural science research which has become possible today is the field of structural, biochemical, functional and behavioural correlates of a variety of psychological or psychiatric disorders. With the availability of techniques for precisely delineating structural, biochemical and metabolic abnormalities in intact animals (including human), it is now possible to explore various higher mental fuctions and their disorders from a biological stand point.

The study of memory mechanism, its neuroanatomical biochemical, physiological and behavioural basis constitutes a vital area of neurological research.

5.10.1 *Drug/substance abuse*

In view of the current global concern in this subject and the specific problems peculiar to India, urgent attention is needed in this field.

5.10.2 *Drug abuse*

Basic studies on drug abuse are needed in certain areas of special relevance to India. Important problems should include:

- Development of animal models to study substances abuse, for example opium, Indian alcoholic liquors, cannabis, etc. need detailed studies as their response may differ from the more thoroughly studied pure compounds like morphine, heroin, cocaine, etc.
- The abuse potential of some new psychotropic drugs developed and introduced in India needs to be studied.
- Morphine and opioid peptides have now been shown to have marked effect on immune system. Their effect on time course of tropical infections has not been investigated so far.
- With drug rules and surveillance becoming more strict in Western countries a new class of drugs of abuse has developed called the Designer's drugs. These are derivatives of drugs of abuse which are not legally banned and can thus escape the enforcement network. It is necessary to have assay procedures for such compounds at least at some centres in the country so that their abuse can be detected before it becomes uncontrollable.

A major problem faced by research workers is the high cost and difficulty in procurement of necessary ligands, narcotic substances, biochemicals, etc. The

National Institute on Drug Abuse, USA supplies these agents free to bonafide investigators through an approved national agency. Immediate steps need be taken to make this facility available to Indian investigators.

5.11 Neuromodelling: Neural implants and neural prosthesis

Active interaction is required between neuroscientists, mathematicians, electronics and computer specialists and experts in biomaterials to develop these exciting areas which promise to provide better understanding of the functioning of the nervous system and help developing implantable devices to replace lost function.

6. HUMAN RESOURCES DEVELOPMENT

It is obvious that there is a paucity of scientists working in the various fields of neuroscience. Those engaged in this activity usually work in isolated disciplinary boundaries, e.g. as neuroanatomists, neurophysiologists, neurochemists, etc. There are very few institutions where groups in all neuroscience disciplines exist. Even these groups are not adequately exposed to modern developments in molecular biology, biotechnology, computer modelling, etc. which are now integral part of frontline research in neuroscience. Hence it is important to create an awareness of these developments, attract younger scientists to neuroscience, provide for multidisciplinary interaction and bring together various groups to learn from each other and develop collaborative research programmes. For this purpose the following activities are recommended:

a) Summer–winter schools conducted by established high quality scientists/groups: Some of the identified individuals and institutions are given below: (This is not an exclusive list but an indicative one.)

i) Neuromorphology: AIIMS, New Delhi
ii) Neuronal membranes: CDRI, Lucknow
iii) Neurotoxicology: ITRC, Lucknow
iv) Neurogenetics: TIFR, Bombay
v) Biology of brain tumours: AIIMS, New Delhi
vi) Animal behaviour: Madurai Kamaraj University, Madurai
vii) Higher nervous activity including Yoga etc: NIMHANS, Bangalore.

b) Seminars and symposia on emerging areas if necessary, supported by international experts

c) Brain storming session: specially in multidisciplinary areas

d) Travelling symposia

e) Training abroad: Young scientists should be selected for training abroad in newly emerging areas not yet developed in India. Some examples are neuroimmunology, neurogenetics and computer modelling of CNS activity. These selected persons could then nucleate this activity in the country.

7. NATIONAL FACILITIES REQUIRED

7.1 There is an urgent need for creating centralized or regional facilities, fully equipped with latest technological advances like nuclear magnetic resonance both imaging and spectroscopy, total body scanner, positron emitting tomography, SPECT, etc. These facilities, no doubt very costly, have opened up completely new vistas to study the morphology, biochemistry, metabolism, circulation and even pharmacokinetics in intact human brain. This would be unimaginable only a few years ago. The investment will pay rich dividends not only for exploring the functions of the normal human brain, but also for the study of a large number of ill-understood diseases of the nervous system.

7.2 Another centralized facility identified was a 'Brain Bank' which could act as a respository of specimens of brains, at all stages of development, at various ages and those afflicted with diseases. These can then be made available to interested researchers. Since the legalization of medical termination of pregnancy the country has a valuable resource of human foetuses, which could be used for studying all aspects of developmental neurobiology. Possibilities of utilizing this for neural transplant work opens up a challenging area of frontline research. Centralized facilities are also necessary for quantitative morphometric studies which would be required for a large number of morphological investigations of the normal and pathological brains.

7.3 Centralized facilities need to be created for procuring and raising special strains/species of genetic mutants required for basic neurobiology research.

7.4 National Information Centre for Neurosciences: There is a need to collect, collate and computerize information regarding current neuroscience activities in the country, procure literature and information at the international level, critically evaluate this data and make it available to neuroscientists in the country.

7.5 Strengthen and support unit of neuroscience in institutions where nuclei of activity of high quality already exists on the lines of advanced centres of COSIST programmes.

ANNEXURES

Annexure 1

RECOMMENDATIONS REGARDING IMPROVEMENT IN EXISTING STATUS OF MEDICAL RESEARCH

1. The condition of investigative medicine is precarious in India. This is no new development. The lack of creativity dates back to hundreds of years. The adventure of Western medicine and political independence produced medical colleges and doctors but their collective contribution to medical knowledge has had little impact on the course of global medicine. Any measures to revive investigative medicine cannot ignore the deep seated nature of the present malady.

2. The reasons for the weakness of investigative medicine are many. A list would include a total alienation from our medical past; poor preparedness in basic sciences such as mathematics, physics, chemistry, etc. when a student enters the medical college; orientation of curricula at undergraduate and postgraduate levels towards passing examinations rather than developing investigative attitudes; selection and promotion of medical teachers on considerations other than competence in research and professional work; frequent transfer of faculty, etc.

3. The Indian Council of Medical Research primarily serves its own institutions and has played a limited role in funding, and working for remedies for the present situation.

4. The remedies must be found at short and long term levels. Both are equally important as one without the other would be less than productive.

4.1 Short-term steps

4.1.1 Seminars on three themes should be organized regionally with investigators of proven ability in medicine and related areas as faculty. The themes suggested are: (a) introduction to medical research (historical, epidemiology, biostatistics, experimental design, medical writing, how to get research funds etc.); (b) common frontiers of medicine and biology; and (c) common frontiers of medicine and engineering. The selection of faculty as well as participants should be done with great care so that the exercise will result in discovery of at least a small number of competent young people who wish to take up medical research seriously. Persons so identified should not only be helped and encouraged to prepare research proposal but also given some incentives to attract them to research.

4.1.2 The participants of the seminars should be assisted by senior investigators constituting the faculty of the seminars in giving formal shape to their proposals which should receive expeditious consideration. The stress should be on young investigations under 40 years and the search should be for a 'spark' in their projects.

4.1.3 Resources should be allocated to arrange for the visits by selected young investigators to spend sometime with institutions. Individuals actively engaged in research, specially to learn newer techniques and technologies.

4.1.4 It is worth considering if some of the selected persons from peripheral institutions may be encouraged to have a 'perceptor/guide' from better endowed institutions.

4.2 Long-term steps

4.2.1 Students must have a much stronger base in mathematics, physics, chemistry and language skills than at present, before they enter the MBBS course.

4.2.2 Undergraduate and postgraduate courses should have less stress of examinations and more emphasis on investigating things for themselves. This may involve literature search, clinical search, experimental electives and so on. Achievements in these should be given as much credit as for grades in examinations.

4.2.3 The selection and promotion of teachers should give as much importance to research capability and achievements as teaching experience, seniority, etc. Prior to recruiting a lecturer, it should be mandatory that he has spent at least two years in a research laboratory, as is done in all advanced countries.

4.2.4 All medical colleges should have adequately equipped research laboratories as also research positions to enable those aspiring to academic careers to work in these laboratories.

4.2.5 Medical colleges should be delinked from government service and be an integral part of a university.

4.2.6 Till a culture for investigative work is created, some seed money and opportunity for members of the medical college faculty be provided to enable them to undergo 'short term' courses in medical research in established research institutes in India or abroad.

4.2.7 Role of various grant giving agencies for promoting medical research be critically evaluated and ways and means found to strengthen these and provide for greater co-ordination amongst them. The question of having one single agency, which is not only for giving grant, but also promote research needs to be debated.

4.2.8 The new agency should encourage medical research in the medical colleges as a matter of policy.

Annexure 2

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ARTICLES

STATISTICAL ANALYSIS OF LINEAMENTS OF GOA, INDIA

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ABSTRACT

A study of the lineaments of Goa identified from aerial photographs is presented here. The shapes of histograms representing direction vs total number and direction vs total length have close similarities. The trended data set was treated statistically to obtain the nonlinear pattern in the form of a cosine wave. Three distinct peaks were found at azimuths of 40–45°, 90–95° and 140–145°, which have peak values of 5.85, 6.50 and 5.80 respectively. These three peaks are correlated with three cycles of tectonic activities occurring in this region, the most prominent being in the E–W direction.

INTRODUCTION

LINEAMENTS are representations of linear physiographic features related to joints, faults, fractures or folds. A study of lineaments gives valuable information for groundwater and mineral exploration and in understanding the tectonic framework of any area. Though the term 'lineament' is used for apparently straight features, it is well known that no geological trend is absolutely straight. Stream diversions, minor faulting and jointing and other features may be present in any given area.

Previous studies of lineaments of Goa[1-4] indicated three major trends NW–SE, NE–SW and ENE–WSW. This study presents a mathematical relationship between the length and direction of lineaments and correlates them with the observed tectonic activities.

MATERIALS AND METHODS

The study area is between 14° 48′ N and 15° 48′ N, and 74° 20′ E and 75° 40′ E along the central west coast of India. It is bounded by the rivers Terekhol in the north and Kali in the south, and by the Sahyadris in the east and the Arabian Sea in the west (figure 1). The lineaments were traced from aerial photographs (scale 1 : 60,000 approx.) and satellite images. In this area 207 lineaments were traced, and their lengths were measured and represented as histograms (figure 2). Field work was carried out to confirm some of these lineaments.

The lineament data have been divided into 5° equidistant intervals in terms of direction. Over 34 intervals were averaged and the average lineaments were subjected to statistical treatment in terms of direction (table 1).

The division of the average lineament data into such equidistant intervals gives rise to the pattern shown in figure 3. It was desired to obtain this nonlinear pattern in the form of a cosine wave to evolve the periods of amplitude from wave movement[5].

$$\chi_{t^\circ} = \bar{X} + R\cos(\omega t + \phi) + \varepsilon t^\circ,$$

where $\bar{X}$ is the average of χ_{t° for $t = 1, 2, 3, \ldots, n$; R the amplitude of the wave; ω the frequency, and ϕ the phase angle.

The above equation can also be written as

$$\chi_{t^\circ} = X + A\cos\omega t^\circ + B\sin\omega t^\circ + \varepsilon t^\circ$$

where A and B are constants and $R = (A^2 + B^2)^{1/2}$ and $\phi = \tan^{-1}(B/A)$. The constants are obtained by the method of least squares.

Because analysis of periods in a data series demands removal of any trend the data were detrended. The lineaments were classified into different intervals and the data were averaged with fluctuations being minimized. The average lineament features were divided by moving average, which contained trend, in order to detrend the data (table 1). The data series was left only with period or cycles in the 'bloom' of lineaments with change in

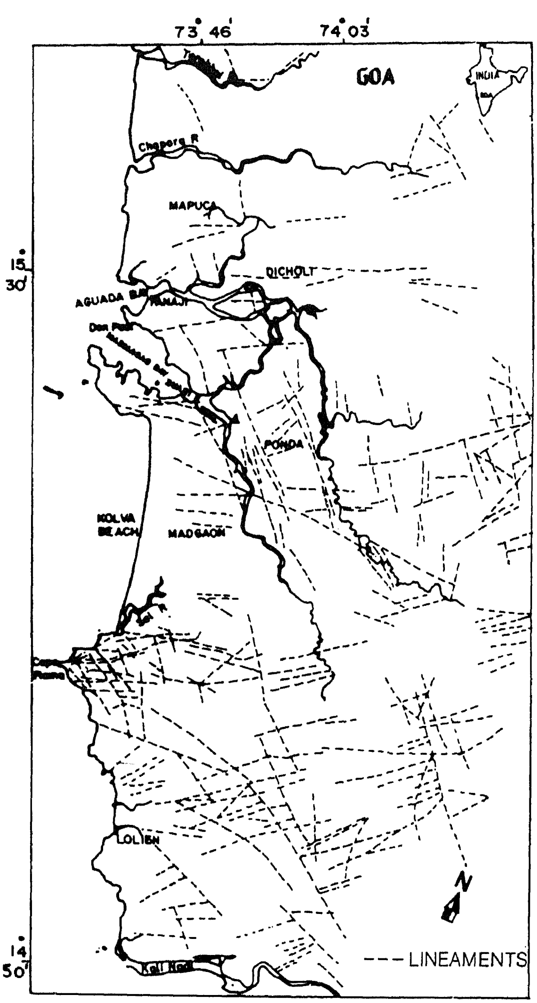

Figure 1. Lineament map of Goa.

direction. The cosine curve which explains the period or cycles of the lineaments was of the form

$$\chi_{t^\circ} = 1.299 + 0.134\cos(1.84\,t^\circ - 79.64\,t^\circ).$$

Also,

$$\chi_{t^\circ} = 1.299 + 0.024\cos(1.84\,t) - 0.131\sin(1.84\,t^\circ)$$

The frequency ω of the curve was found to be 1.8487; $\omega = 2\pi f/n$, where f is the number of peaks in the curve and n the number of observations. The value $\omega = 1.8487$ was found for $f = 10$.

RESULTS AND DISCUSSION

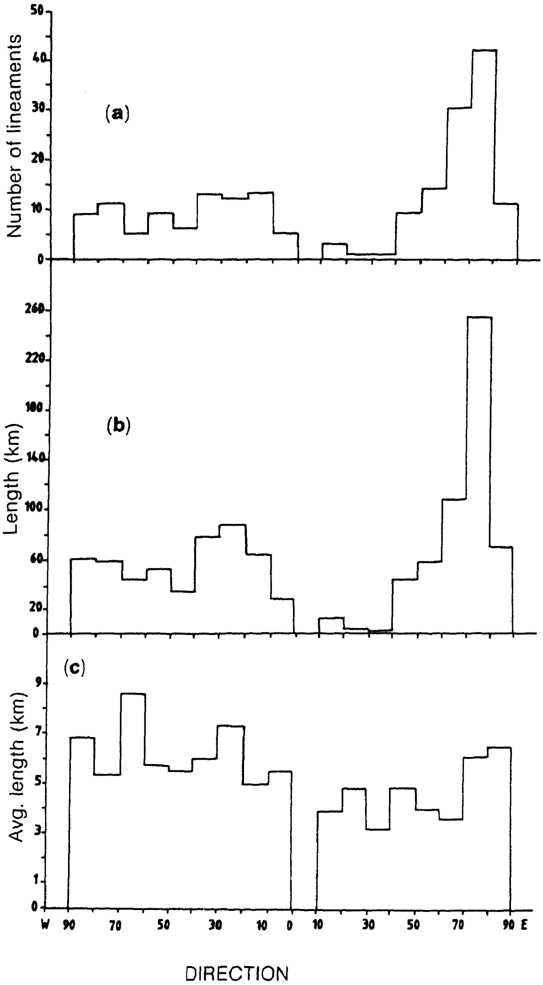

Figure 2. Histograms: (**a**) number of lineaments in 10° direction intervals, (**b**) total length of lineaments, and (**c**) average length of lineaments.

In the study area 207 lineaments were measured. The lineaments have a total length of 1095.96 km spread over an area of 4721.04 km^2, giving an average lineament density of 0.043/km^2.

Three kinds of histograms were drawn[6] (figure 2): (i) number of lineaments in each 10° against the segment bearing, (ii) total length of all lineaments in each 10° segment against the segment bearing, and (iii) the average length of lineaments in each 10° against the segment bearing compared with the average length of all lineaments in the area.

Figure 2 reveals the following. Generally, the histograms for total number of lineaments (figure 2a) and total length of lineaments (figure 2b) show fairly close similarities in shape. A slight fall in lineament

Table 1 *Statistical treatment on data for lineaments of Goa*

Direction interval (deg)	Average lineament	Moving average lineament	Detrended lineament
10–15	4.38	4.38	1.00
15–20	3.72	4.05	0.92
20–25	4.86	2.97	1.64
25–30	3.24	3.24	1.00
30–35	2.59	2.59	1.00
35–40	3.18	2.69	1.18
40–45	5.85	3.14	1.86
45–50	4.59	3.32	1.38
50–55	2.75	3.25	0.85
55–60	4.60	3.39	1.32
60–65	3.59	3.41	1.05
65–70	3.86	3.44	1.12
70–75	6.04	3.65	1.66
75–80	5.45	3.78	1.44
80–85	5.60	3.89	1.44
85–90	3.15	3.85	0.82
90–95	6.50	4.00	1.48
95–100	1.62	3.87	0.42
100–105	4.24	3.89	1.09
105–110	5.62	3.98	1.41
110–115	7.39	4.14	1.79
115–120	5.40	4.12	1.31
120–125	4.84	4.23	1.14
125–130	5.33	4.27	1.25
130–135	4.59	4.28	1.07
135–140	7.41	4.41	1.68
140–145	5.80	4.46	1.30
145–150	6.85	4.54	1.41
150–155	7.29	4.64	1.57
155–160	13.86	4.94	1.40
160–165	6.48	4.97	1.30
165–170	5.66	5.07	1.13
170–175	5.98	5.02	1.19
175–180	5.46	5.02	1.08

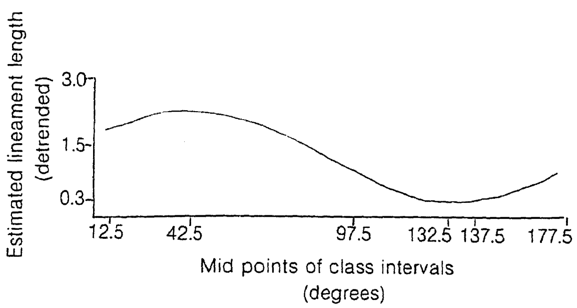

Figure 3. Cosine wave curve obtained from data for lineaments of Goa.

number is seen between 20 and 30° W for which a corresponding peak occurs in the length histogram. This may be explained as being caused by greater lengths of a smaller number of lineaments. The number of lineaments in this zone (20–30° W) is 12, with a total length of 88 km. The 'average length' histogram (figure 2c) and the 'total number' histogram show that in the direction internal 70–80° E there is an increase in average length of lineament and a greater concentration of lineaments. Hence the 'total lineament length' histogram shows a distinct and sharp peak in this region. But the 'average length of lineament' histogram does not closely conform to the shape of the 'total number of lineament' histogram in the other direction intervals. To some extent the 'average length' histogram has an apparent increase whereas the 'total number' histogram shows an apparent decrease in concentration of lineaments. Hence the 'total lineament length' histogram tends to have some change in the pattern outside the 70–80° E interval compared to the pattern in the 'total number of lineaments' histogram.

The cycle in the data was observed to be 10, i.e. after every ten direction intervals a peak in the average lineament feature occurs. In cos $(\omega t + \phi)$, because of negative ϕ the $t = \phi/\omega$ value corresponds to about 43.07°, at which the cosine curve vanishes. This value of 43.07° lies in the 40–45° direction interval in which the first peak with average lineament length of 5.85 is observed. The next peak, of 6.5, occurs in the 90–95° interval, followed finally by a peak of 5.80 in the 140–145° interval. The fit therefore seems to be in reasonable agreement with the observed data.

The present results indicate that the moving average of lineaments shows an increasing trend with direction. The highest peak value of 6.5 (90–95°) corresponds to the E–W trending round hinged folds. The other two peak values, 5.85 and 5.80, correspond to the NW–SE and the NW-plunging folds respectively.

CONCLUSION

In conclusion it may be stated that tectonic activities were maximum in the E–W direction, and less in the NW–SE and the NE directions. These results corroborate earlier studies[7] which revealed that the major parts of Goa witnessed three cycles of folding of varying intensities during the Archaean.

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9 January 1989; Revised 23 March 1989

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ANNOUNCEMENTS

Success and Creativity in Pharmaceutical Research and Development

Place: Royal College of Physicians, London
Date: 30 April to 1 May 1990
Contact: Dr Renata Duke
IBC Technical Services Ltd.
Bath House (3rd Floor)
56 Holborn Viaduct,
London EC1A 2EX, UK

Bisphosphonates — Current Status and Future Prospects

Place: Royal College of Physicians, London
Date: 21/22 May 1990
Contact: Dr Renata Duke
IBC Technical Services Ltd.
Bath House (3rd Floor)
56 Holborn Viaduct
London EC1A 2EX, UK

Autoimmunity — New Targets and Therapeutic Approaches

Place: Royal College of Physicians
Date: 5/6 June 1989
Contact: Dr Renata Duke
IBC Technical Services Ltd.
Bath House (3rd Floor)
56 Holborn Viaduct
London EC1A 2EX, UK

Twentyfifth Course on Management of R and D Systems

Place: Hyderabad
Date: 12–17 March 1990
Contact: The Programmes Officer
Administrative Staff College of India
Bella Vista, Hyderabad 500 049

National Congress on Biotechnology

Place: Hyderabad
Date: 16–18 March 1990
Theme: Biotechnology for Human Welfare
Contact: Prof. P. P. Reddy
Director
National Congress on Biotechnology
Institute of Genetics
Hospital for Genetic Diseases
Begumpet, Hyderabad 500 016

CYSTEINE PROTEASE INHIBITORS FROM BANANA (*MUSA PARADISIACA*)

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ABSTRACT

Inhibitors of cysteine proteases were found in ripened and unripened banana (*Musa paradisiaca*). Extract from unripened banana inhibited the proteolytic activities of papain, ficin and bromelain. Extract from ripened banana inhibited the proteolytic activities of papain and ficin only. Both ripened and unripened banana extracts inhibited caseinolytic and amidolytic activities of papain. The inhibitory activity of ripened banana extract was stable to extreme acidic pH and neutral pH and unstable to extreme alkaline pH. The inhibitory activity of ripened and unripened banana extracts was stable after heating to 100°C for 10 min.

INTRODUCTION

CYSTEINE protease inhibitors from chicken egg white[1], human serum[2], bovine colostrum[3] and microorganisms[4] have been reported. Papain, a plant cysteine protease evolutionarily related to animal cysteine proteases, was used in these studies to identify the inhibitors. While the mammalian inhibitors of cysteine proteases have been extensively investigated, studies on similar factors from plant sources are limited[5,6]. In view of this an attempt was made to identify inhibitors of papain from plant sources. This communication reports the presence of cysteine protease inhibitors in ripened and unripened banana (*Musa paradisiaca*) and some of their properties.

MATERIALS AND METHODS

Ripened and unripened bananas (*Musa paradisiaca*, *Musa cavendish*, *Musa sapientum* and Nendran) were procured from local sources. Twice-crystallized papain (EC 3.4.22.2), twice-crystallized ficin (EC 3.4.22.3), bromelain (EC 3.4.22.4), *N*α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), bovine serum albumin and casein were obtained from Sigma Chemical Company, USA. Other reagents were analytical grade commercial chemicals.

Preparation of banana extract

Twenty-five g of ripened or unripened banana without the outer skin was homogenized in 25 ml of 0.02 M borate buffer, pH 7.6. The homogenate was centrifuged at 10,000 *g* for 20 min at 4°C. The supernatant was collected and tested for inhibitory activity. Protein content of the supernatant was measured by the method of Lowry *et al.*[7] using bovine serum albumin as standard.

The activities of papain, ficin and bromelain were measured with casein as substrate by a method modified from Kunitz[8]. The assay system consisted of 100 μmol of borate buffer pH 7.6, 5 μmol of EDTA, 10 μmol of β-mercaptoethanol, 10 mg of casein and water in a volume of 1.8 ml. The reaction was started by the addition of 0.2 ml enzyme solution. After a 15 min incubation at 37°C, the reaction was arrested by adding 3 ml of 5% trichloroacetic acid. After standing at room temperature for 30 min, the contents were centrifuged at 2000 *g* for 10 min. The concentration of trichloroacetic acid-soluble peptides was determined in 1.0 ml of the clear supernatant by the method of Lowry *et al.*[7] Under the assay conditions 8 μg of papain, 21.5 μg of bromelain and 25 μg of ficin liberated peptides equivalent to an absorbance of 0.60 from casein. Papain was also assayed using BAPNA as substrate by a method modified from Erlanger *et al.*[9] The assay system consisted of 100 μmol of borate buffer pH 7.6, 5 μmol of BAPNA, 5 μmol of EDTA and 10 μmol of β-mercaptoethanol in a volume of 2.8 ml. The reaction was started by addition of 0.2 ml of enzyme solution. After a 30 min incubation at 37°C the reaction was arrested by adding 1 ml of 30% acetic acid. The yellow colour that developed was measured at 410 nm. Under the assay conditions 54 μg of papain was used to get an absorbance of 0.60.

Estimation of papain inhibitory activity

Suitable aliquots of banana extracts were included in the assay system described above. Reduction in papain activity was the measure of inhibition. One unit of inhibitory activity (in the caseinolytic and amidolytic methods) was the amount that suppressed hydrolytic activity by one absorbance unit.

Effect of pH on the inhibitory activity of banana

Ripened banana extract (0.1 ml, containing 400 μg protein) was incubated with 0.1 ml of different buffers for 72 h at 4°C: 0.1 M HCl (pH 1.0), 0.1 M HCl/KCl (pH 2.0), 0.1 M acetate (pH 4.5), 0.1 M borate (pH 7.0 and 8.0), 0.1 M bicarbonate (pH 10.0), and 0.2 M NaOH (pH 12.5). After the incubation 0.4 ml of 0.2 M borate buffer pH 7.6 was added and the extract assayed for inhibitory activity against caseinolytic activity of papain.

Effect of temperature on the inhibitory activity of banana

Four ml of ripened or unripened banana extract was subjected to heat treatment at 70, 90 and 100°C for 10 min. After cooling, aliquots were assayed for papain inhibitory activity.

Controls without banana extract were run simultaneously in all cases.

RESULTS

Crude extracts of ripened and unripened banana were found to inhibit several cysteine proteases. Data for inhibition of hydrolytic activities of three cysteine proteases by banana inhibitor are presented in table 1. The caseinolytic and amidolytic activities of papain were maximally inhibited by ripened banana inhibitor. The ripened banana extract showed moderate inhibition of caseinolytic activity of ficin and had no action against caseinolytic activity of bromelain. Unripened banana inhibited the caseinolytic activities of papain and ficin to some extent and bromelain weakly. The inhibition of caseinolytic activities of papain and ficin by ripened banana extract were much more than that of unripened banana extract. However, the latter showed inhibition of caseinolytic activity of bromelain, which was not shown by ripened banana inhibitor. Inhibition of amidolytic activity of papain by both ripened and unripened banana extracts was two times more than inhibition of caseinolytic activity.

The papain inhibitory activity of ripened banana extract as a function of concentration is shown in figure 1. Inhibition of caseinolytic activity of papain was linear up to 33%; beyond this the inhibition was not linear and sluggish, and maximum inhibition obtained at very high inhibitor concentration was only about 80%. In contrast, inhibition of amidolytic activity of papain by ripened banana inhibition was linear up to 70% and complete inhibition was obtained at high inhibitor concentration. Amount of protein (inhibitor) required to cause 50% inhibition was 600 μg for caseinolytic activity (calculated by extrapolation of the linear range) and 300 μg for amidolytic activity.

The stability of inhibitory activity of ripened

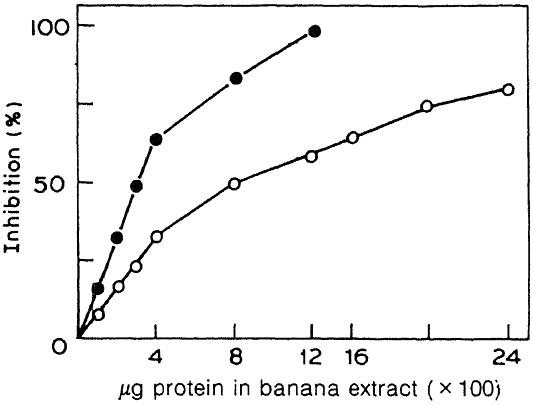

Figure 1. Inhibition of hydrolytic activities of papain by ripened banana extract. ○---○, Inhibition of caseinolytic activity; ●---●, inhibition of amidolytic activity.

Table 1 *Inhibition of proteolytic activity of cysteine proteases by banana extract*

	Inhibition (inhibitory units/ml)			
	Papain		Ficin	Bromelain
Extract	Caseinolytic activity	Amidolytic activity	Caseinolytic activity	Caseinolytic activity
Ripened banana	2.00	4.00	1.00	0.00
Unripened banana	1.20	2.40	0.60	0.30

banana inhibitor to acidic, neutral and alkaline pH was studied and the result is shown in figure 2. The inhibitory activity was stable to acidic and neutral pH and was unstable to alkaline pH. At pH 10 there was 30% loss of activity of inhibitor and complete loss of inhibitory activity was observed at pH 2.5.

The inhibitory activity in ripened and unripened banana was highly heat-stable. No loss of inhibitory activity was noticed even after exposure to 100°C for 10 min.

Papain inhibitory activity was also found in plantain (*M. cavendish*), vegetable banana (*M. sapientum*) and Nendran banana.

DISCUSSION

This report establishes the presence of cysteine protease inhibitors in plants, especially in a storage organ like the fruit. It is known that cysteine protease inhibitors of animal origin are inactive against bromelain[10]. Unlike the animal cysteine protease inhibitors, the unripened banana inhibitor was active against bromelain. It has been reported that the magnitude of inhibition of serine proteases by plant tuber inhibitors depends on the substrate employed[11]. The higher inhibition by banana extract of BAPNA hydrolysis by papain compared to casein hydrolysis suggests that magnitude of inhibition of cysteine proteases by plant inhibitors also depends on the substrate used.

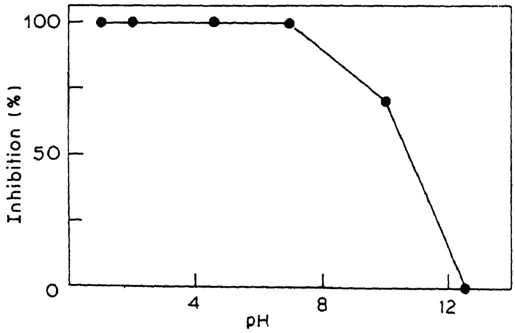

Figure 2. Effect of pH on stability of banana inhibitor of papain caseinolytic activity.

The extreme heat stability and alkaline-sensitive nature of banana inhibitors indicate that the factors responsible for inhibition may be thiol-dependent proteins which probably inhibit the activity of cystein proteases by a disulphide exchange mechanism[1,12].

The exact biological role of cysteine protease inhibitors in plants is not known at present. But the low inhibitor levels in unripened banana compared to ripened banana suggests that the synthesis of cysteine protease inhibitor increases as ripening advances.

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SHORT COMMUNICATIONS

FREELENGTHS IN RELATION TO THE MOLECULAR INTERACTIONS IN LIQUIDS

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WEISSLER *et al.*[1] and Lagemann *et al.*[2] reported data on ultrasonic velocities, densities and viscosities of some diols at 30°C and chlorotrifluoroethylene at 60°C. Suryanarayana and Pugazhendhi[3] calculated internal pressure, and its variation was correlated with molecular weight. The method of calculation of some thermodynamical parameters in relation to intermolecular association were reported[4, 5]. Adiabatic compressibility and intermolecular freelengths are important quantities in determining complex formation of relatively stronger cohesion between molecules. Adiabatic compressibility is calculated using

$$\beta_S = 1/c^2 \rho, \qquad (1)$$

where c and ρ are ultrasonic velocity and density. The intermolecular freelength is then estimated using

$$L_f = K \beta_S^{1/2}, \qquad (2)$$

where K are temperature-dependent constants reported earlier. Jacobson[6] reported these constants K for a range of temperatures. According to him, if c is in m/s and ρ in g/ml then L_f will be in Å.

Considering the importance of uniformity in system of units and the values of K for any intermediate temperature, the data given by Jacobson are fitted to an equation,

$$K = K_0 + K_1 t, \qquad (3)$$

where K_0 and K_1 are best fit parameters and t is in °C. The reproduction of the data is within an error of 1%. The parameters K_0 and K_1 are shown in table 1 in the SI system of units. Now with the values of K_0 and K_1 it is easy to estimate L_f at any temperature in the range 0–50°C. Considering the linear fit it is also felt that the values of K_0 and K_1 are useful even at slightly higher temperatures, i.e. up to 75°C. Above 75°C most organic liquids begin to vaporize and hence the use of the values K_0 and K_1 in the estimation of L_f at any temperature below 75°C up to 0°C is understood.

Table 1 *Values of K and the best fit parameters K_0 and K_1*

Temp. (°C)	From ref. 6	Present work K	Present work K (SI) ($\times 10^{-6}$)
0	588	591	1.87
10	604	604	1.91
20	618	617	1.95
25	625	623	1.97
30	631	629	1.99
40	642	642	2.03
50	652	655	2.07
	K_0	591	1.87
	K_1	1.28	0.004

The study of intermolecular freelength is important in understanding molecular interactions. In a binary system, while there is a concentration variation of one component, the presence or absence of any cohesive or repulsive forces may be predicted by the calculation of intermolecular freelength L_f. If L_f increases with concentration change then one may say that a repulsive interaction is predominant between the components. If L_f decreases with concentration change then one may conclude that an attractive interaction is predominant between the components. If there is no appreciable increase or decrease in L_f then one may conclude that both attractive and repulsive interactions exist and are equal and opposite. To demonstrate the above let us consider the data on diols at 30°C and chlorotrifluoroethylene[3]. The adiabatic compressibility and intermolecular freelengths are computed and are presented in table 2. From part A of the table it is clear that there is no appreciable change in L_f with increase in molecular weight from ethylene glycol to pentaethylene glycol. Hence one may conclude that even if there are any interactions the repulsive and cohesive parts are equal in magnitude and opposite in nature. A similar trend is seen in part B of the table. In contrast, in part C, there is gradual decrease in intermolecular freelength with increase in molecular weight. Hence one may expect an attractive cohesion between the components in the mixture. Because of the high electronegativity of the fluorine atoms there may be localization of δ^+ centres on the carbon atoms and δ^- centres on the electronegative atoms. Thus there occurs a strong cohesion and hence shorter and

Table 2 *Molecular weight, intermolecular freelength and related parameters for diols and chlorotrifluoroethylenes*

	Molecular Weight	c ($\mathrm{m\,s^{-1}}$)	$\rho \cdot 10^3$ ($\mathrm{kg/m^{-3}}$)	$\beta_{ad} \cdot 10^{-10}$ $\mathrm{N^{-1}\,m^2}$	L_f (Å)	
					a	*b*
A: (30°C)						
Ethylene glycol	62.1	1643	1.107	3.346	0.3650	0.3640
Diethylene glycol	106.1	1568	1.109	3.668	0.3821	0.3811
Triethylene glycol	150.1	1593	1.116	3.531	0.3750	0.3739
Tetraethylene glycol	194.2	1580	1.116	3.589	0.3780	0.3770
Pentaethylene glycol	238.2	1580	1.117	3.586	0.3779	0.3769
B: Polyethylene glycols (30°C)						
	200	1592	1.117	3.532	0.3750	0.3740
	300	1578	1.118	3.592	0.3782	0.3772
	400	1576	1.118	3.601	0.3787	0.3776
	600	1570	1.118	3.629	0.3801	0.3791
C: Chlorotrifluoroethylenes[c] (60°C)						
	525	731	1.815	10.311		0.6775
	563	747	1.831	9.787		0.6601
	577	753	1.838	9.595		0.6536
	664	785	1.869	8.683		0.6217
	686	794	1.877	8.451		0.6134
	713	804	1.885	8.207		0.6045
	727	803	1.887	8.219		0.6049
	789	811	1.901	7.998		0.5967
	846	823	1.909	7.734		0.5868
	886	831	1.914	7.566		0.5804
	894	830	1.920	7.560		0.5802
	911	836	1.919	7.456		0.5762
	948	839	1.926	7.376		0.5730

[a] Calculated using K values given by Jacobson.
[b] Calculated using the K values from eqn. (4).
[c] At 60°C K values are not available from ref. 6.

shorter intermolecular freelengths with increase in molecular weight. The values of freelength were calculated using K_0 and K_1 at 60°C. The predictions of the intermolecular interactions agree with those predicted by other methods[3]. The typical values of L_f for the above case are around 0.6 Å and even though these values are very small compared to the typical size (nearly 5 Å) of the molecules under study, the estimates of L_f give a picture of the presence of any intermolecular cohesion.

From the above discussion it is clear that the fitting of the Jacobson's temperature-dependent constants K are necessary to estimate L_f at all temperatures below 75°C up to 0°C. It may also be concluded that if there is any repulsive force there would be an increase in the value of intermolecular freelength. Both experimental and theoretical studies are in progress to confirm such an important role of the intermolecular freelength in molecular interaction.

The author thanks Prof. K. S. Chandrasekaran for useful discussions.

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PETROLOGY OF THE POST-TECTONIC GRANITES FROM GUNDLUPET, SOUTHERN KARNATAKA

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THE South Indian craton is transected by lineaments, some of which are taphrogenic in nature[1]. These lineaments host many granite and syenite plutons of possible Pan-African age[2]. This paper records for the first time the occurrence of two granite bodies along the Moyar lineament near Gundlupet (lat. 11° 45′ N, long 76° 43′ E). The area around Gundlupet forms a part of the Sargur high grade terrain in southern Karnataka[3] and lies just north of the N70°E trending Moyar lineament. The lithologies include metasediments (pelites, calc-silicate rocks, quartzites, BIF and manganiferous horizons), amphibolites, various components of ultramafic-layered complex, and gneisses of various ages. Grey phase of the Peninsular gneiss is dated[4] 2.85 byr. The lithologies point to upper amphibolite facies metamorphism and show imprints of four major deformational episodes.

The granites of the present study occur as small bodies intruding into the Peninsular gneisses near Kodihalli and Mallayanpura. The contact of the granite body with the country rocks is marked by mylonite zones.

The medium- to fine-grained and light-pink granite shows hypidiomorphic granular texture and has the assemblage: quartz–plagioclase–K-feldspar–clinopyroxene–hornblende.

Quartz is generally anhedral with undulose extinction. Plagioclase (An 10–15%) is a minor phase and occurs as subhedral grains with moderately developed albite twinning, and is weakly sericitized. K-feldspar is anhedral with crosshatched twinning and occasionally contains string perthite. Clinopyroxene is feebly pleochroic and occurs as subhedral grains. Strongly pleochroic hornblende occurs around clinopyroxene with fused margins. The accessories such as zircon and apatite are found as inclusions within feldspars. Allanite is metamict, golden-yellow, and exhibits zoning. Sphene occurs both as ovoidal grains and as grains rimming magnetite. Calcite is subhedral and shows well-developed rhombohedral cleavages. Magnetite is generally seen as granules in association with mafic phases and sphene.

Chemical analysis of the granites was done using XRF at the Department of Geology, University of Glasgow, Glasgow, UK. The analytical data for major and trace elements are given in table 1. The chemistry indicates that the granite is quartz normative. However, the presence of normative diopside (up to 15.5%) indicates alkaline nature. The granites show relatively low SiO_2 and Al_2O_3 and higher Na_2O and K_2O (around 10%) when compared to the granites (*s.s*) reported elsewhere. The presence of clinopyroxene, amphibole, allanite, sphene and magnetite in the crystallizing assemblage is characterstic of I-type granites[5]. In the SiO_2–log (K_2O/MgO) diagram[6] (not given), the granites fall in the alkali post-orogenic granite region. When $Al_2O_3 + CaO + \text{total alkalies}/Al_2O_3 + CaO - \text{total alkalies}$ is plotted against SiO_2 (ref. 7) (diagram not given), the granites fall in the alkali granite region.

Table 1 *Major and trace element composition of granites from Gundlupet*

Major elements

	24	13a	Normative composition		
SiO_2	65.62	60.17	Qz	15.65	5.34
TiO_2	0.32	0.23	Or	42.72	33.36
Al_2O_3	14.93	12.72	Ab	20.65	33.01
Fe_2O_3*	3.79	6.01	An	8.43	5.56
MnO	0.02	0.21	Di	4.26	15.50
MgO	1.84	2.31	Wo	—	3.60
CaO	3.21	6.62	hy	3.81	—
Na_2O	2.44	3.86	He	2.75	3.36
K_2O	7.23	5.56	Mt	—	—
P_2O_5	0.33	0.74	Il	0.61	0.46
Total	99.83	99.05	Ap	0.78	1.68

Trace elements (ppm)

	24	13a
V	47	ND
Ni	—	5
Cr	—	11
Cu	—	2
Zn	91	183
Ga	17	21
Rb	147	158
Sr	811	884
Y	47	57
Zr	281	61
Nb	19	13
Ba	1665	1057
La	78	246
Ce	246	458
Pb	66	23
Th	198	73
U	5	ND

*Denotes total iron.
ND, not determined.

In the alkali–silica diagram[8], the granites fall well within the alkaline region.

The granites contain higher Rb, Ba and Sr. Rb and Ba are partitioned in K-bearing minerals such as K-feldspar and biotite. The high Rb and Ba correlate well with the high modal K-feldspar. Sr is dominantly partitioned in plagioclase and other Ca-bearing minerals such as apatite and clinopyroxene. The granites of the present study, though containing higher Sr, have low modal plagioclase. To account for high Sr another mineral phase is required. Indeed the granites of the present study contain abundant clinopyroxene which have high Kd value for Sr.

The high field strength elements (HFS) such as Nb, Zr and Y are considered to be immobile during secondary processes and their abundance in the granite of the present study indicates alkaline affinity. The high concentration of LREE (La and Ce) is the unique feature of these granites. In the absence of analytical data on all the HREE the La/Y ratio can be taken as an indicator of LREE/HREE, as Y behaves like HREE[9]. The average La/Y ratio is 2.98, which is comparable with the reported ratios for the alkaline granites of Kerala[2]. The enrichment of both compatible (Ba and Sr) and incompatible (Rb) elements and low La/Y ratio suggest that the granitic melt has undergone only a limited fractionation. The high K/Rb ratio indicates a Rb-depleted source for the magma. The other compositional features such as low U content and very high Th/U ratio (compared to an estimated crustal average Th/U of ~3.8 (ref. 10) suggest that the source material was strongly depleted in U relative to Th. Such Rb and U depletion suggests a possible lower crustal granulitic source[11]. Melting of anhydrous granulites requires higher temperatures and pressures[12] and the rocks formed by these melts are relatively poor in normative quartz. Furthermore, near-minimum melts derived from rocks with high CaO will be generally more potassic. The presence of CO_2-rich fluids in granulites[13] will cause partial melts to become more alkaline. Thus, based on field and geochemical data, a lower crustal granulitic source is suggested for the granites of Gundlupet.

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TRITERPENES FROM THE LEAVES OF *RHUS ALATA* THUMB.

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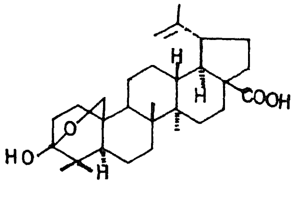
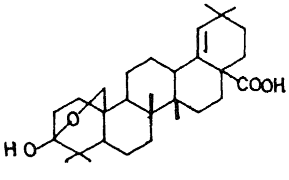
IN continuation of our phytochemical investigations on *Rhus alata* Thumb.[1], we now report the isolation and characterization of triterpenes benulin (I) and semimoronic acid (II). Benulin (I) has been reported earlier by Ionescu *et al.*[2] Semimoronic acid (II) has been reported in nature from only *Rhus semialata*[3] and it seems to be a characteristic phytoconstituent of *Rhus* species. Therefore, semimoronic acid (II) may be useful as a chemotaxonomic marker to establish interspecific relationships in the genus *Rhus*.

Rhus alata Thumb. was collected from Pachunga University College, Aizawl, Mizoram, and identified by Dr W. Husain, Department of Botany of this Uni-



(I)



(II)

versity. A voucher specimen was submitted to the University Herbarium (voucher number Husain–33701).

The dried and powdered leaf material of *R. alata* (1 kg) was defatted with light petrol and extracted with EtOH. The EtOH extract was concentrated and the residue was refluxed with light petrol and benzene, and treated with boiling water. Since the petrol-soluble fraction of EtOH extract and the straight petrol extract show similar components on TLC, they were mixed together. The combined petrol-soluble fraction was chromatographed over a silica gel column. The column was run with light petrol. The fractions eluted with light petrol were crystallized from hexane to give white crystals of RA5 (100 mg, m.p. 129°C). It was found to be dimethylterepthalic acid. Then the column was eluted with petrol–benzene (9:1–1:1), which gave inseparable mixtures of urushiol derivatives. Elution with benzene and benzene–EtOAc (9:1, 8:2) gave a greenish mass of chromatographically comparable components, which solidified on trituration with $CHCl_3$–EtOH. The solid mass, after repeated crystallization from $CHCl_3$–MeOH, gave two isomeric products, RA9 (50 mg, m.p. 283–284°C, R_f 0.56, benzene–acetone 5:1) and RA10 (60 mg, m.p. 260–261°C, R_f 0.48 benzene–acetone 5:1). RA9 was characterized as benulin (I) and RA10 as semimoronic acid (II).

RA9: ^{1}H NMR ($CDCl_3$), δ: 4.72 and 4.62 (1H, *br s* each, $=CH_2$), 4.24 and 3.72 (1H, AB*d* each, $J = 8$ Hz, $-CH_2O-$), 1.66 (3H, *br s*, vinylic CH_3), 1.00 (3H, *s*, CH_3), 0.94 (6H, *s*, $2 \times CH_3$), 0.84 (3H, *s*, CH_3). MS *m/z* (rel. int.): 470 (M^+, 26%), 455(1), 452(2), 439(2), 424(9), 409(2), 397(5), 313(13), 223(18), 205(20), 203(10), 201(13), 189(34), 187(34), 175(26), 163(24), 161(20), 159(19), 155(15), 133(34), 121(50), 119(53), 109(68), 107(60), 105(66), 44(100).

RA10: ^{1}H NMR ($CDCl_3$) δ: 5.16 (1H, *brs*, $=CH-$), 4.28 and 3.72 (1H, AB *d* each, $J = 8$ Hz, $-CH_2O-$), 0.98–0.92 (12H, overlapping *s*, $4 \times CH_3$), 0.84 and 0.72 (3H *s* each, $2 \times CH_3$). MS *m/z* (rel. int.): 470 (M^+, 15%), 452(4), 426(70), 424(41), 411(8), 409(8), 397(9), 246(10), 236(55), 203(32), 191(15). 190(80), 189(68), 175(46), 163(100), 133(22), 121(27), 119(38), 109(30), 107(35), 105(40).

The authors are grateful to Prof. W. Husain, Department of Botany, A.M.U., Aligarh, for identification of the plant and to Dr M. Husain, Pachunga University College, Aizawl, Mizoram, for procuring the plant material. One of us (MP) is thankful to CSIR, New Delhi, for financial assistance.

13 February 1989; Revised 1 November 1989

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TWO NEW SPECIES OF *PSEUDOCERCOSPORA* FROM INDIA

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DURING a survey for plant parasitic fungi, two interesting foliicolous hyphomycetes were collected. These fungi proved to be two new taxa of species rank. They are described below:

Pseudocercospora tephrosiae A. N. Rai *et* Kamal sp. nov.

Maculae pleraeque epigenosae, interdum in superficie inferiori, parvae, dispersae per totam superficiem folii, atro-brunneae; coloniae pleraeque epiphyllosae et raro hypophyllosae, sericae, velate brunneae;

mycelium hypharum immersum, septatum, angustum, ramosum; stromata bene formata, immersa, pseudoparenchymatosa, fusce olivacea, 27.6 μm diametro; conidiophori caespitosi, parvi (in fasciculis densis), macronematosi, mononematosi, suberecti, vix flexuosi, aliqui geniculati, usque 3-transverse septati, raro ramosi, cum parietibus laevibus, pallide vel moderate olivacei, 16.0–59.8 × 3.5–6.9 μm; cellulae conidiogenosae integratae, terminales, polyblastae, sympodiales, denticulatae, cum denticulis stipatis brevibus et acutis vel late conoidibus, cum cicatricibus spissis; conidia simplicia solitaria, arida, acropleurogenosa, pallide olivacea, cylindrata vel obclavatocylindrata, cum parietibus laevibus, 2–6 transverse septata, cum apice subacuto vel obtuso et basi obconicotruncata vel subtruncata et hilis spissis, 20.7–64.4 × 3.5–4.6 μm.

Infection spots predominantly epigenous sometimes on the undersurface, small, distributed all over the leaf surface, blackish brown; colonies predominantly epiphyllous and rarely hypophyllous, velvety, dull brown; mycelium of hyphae immersed; stromata pseudoparenchymatous, dark olivaceous, 27.6 μm in diameter; conidiophores caespitose, small (in loose) to large (in dense) fascicles, macronematous, mononematous, suberect, somewhat flexous, some geniculate, up to 3-transversely septate, rarely branched, smooth-walled, light- to mid-olivaceous, 16.0–59.8 × 3.5–6.9 μm; conidiogenous cells integrated, terminal, polyblastic, sympodial, denticulate, with short pointed to broad conoid closely set denticles with thickened scars; conidia simple solitary, dry acropleurogenous, light-olivaceous, cylindric to obclavatocylindric, smooth-walled, 2–6-transversely septate, with subacute to obtuse apex and obconicotruncate to subtruncate base and thickened hila, 20.7–64.4 × 3.5–4.6 μm (figure 1).

On living leaves of *Tephrosia purpurea* (L.) pers. (Fabaceae); September, 1979; Gorakhpur (Gorakhpur South Forest Division); leg. A. N. Rai, KR 370, type IMI 246396.

A survey of the literature shows that many *Pseudocercospora* species have been reported on the host family[1-5]. However, of all the species of *Pseudocercospora* described so far, the present collection comes close only to *P. pantoleuca* (Sacc.) Deighton[1]. For justifying the distinct identity of the proposed taxon, a comparative account of the morphological characters of the two is given in table 1.

Table 1 reveals that the present collection resembles *P. pantoleuca* in stroma, conidiophores and conidia to some extent only. But it differs from the latter in having darker stroma; distinctly cicatrized, comparatively longer and much wider condidiophores and shorter conidia bearing thickened hila. Besides, the proposed species also differs from *P. pantoleuca* in having predominantly epigenous spots and predominantly epiphyllous colonies as against amphigenous spots and amphiphyllous colonies distributed on both the leaf surfaces in *P. pantoleuca*.

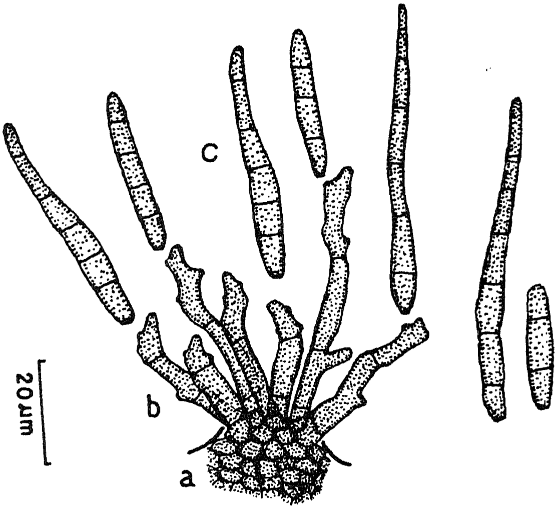

Figure 1. *Pseudocercospora tephrosiae* A. N. Rai et Kamal sp. nov. **a,** Stroma; **b,** conidiophores; **c,** conidia.

The present collection therefore warrants description as a new species. It is also noteworthy that no species of *Pseudocercospora* is reported on the host genus in question.

Pseudocercospora tinosporae A. N. Rai *et* Kamal sp. nov.

Maculae amphigenae, perparvae vel majores, necroticae, nervisequae, irregulares, griseobrunneae, interdum halone viridi circumcinctae; coloniae amphiphyllae, ad partem necroticam limitatae, griseobrunneae; hyphae immersae, septatae, glabrae, ramosae; stromata bene evoluta, irregularia, pseudoparenchymatica, medio-olivacea, 9.2–23.0 μm diametro; conidiophora macronemata, mononemata, caespitosa, dense conferta, usque 3-septata, ramose, suberecta, apices versus subflexuosa, pallide olivacea, 11.5–59.8 × 3.5–4.6 μm; cellulae conidiiferae integratae, terminales, polyblasticae, sympodiales, denticulis brevibus vellatis subacutis interdum dense confertis ornatae;

Table 1 *Comparative morphological characters of P. pantoleuca and P. tephrosiae*

		Conidiophores			Conidia		
Species	Stromata	Structure	Colour	Size (μm)	Structure	Colour and septation	Size (μm)
P. pantoleuca	Small, 15–25, composed of a knot of rather pale oliv. swollen hyphae	In dense fascicles, smooth, straight or sinuous, sometimes slightly geniculate, usually simple but occasionally with a short lateral branch, continuous or the longer ones septate, conidial scars inconspicuous	Rather pale oliv.	15–40 long. occasionally up to 52 long 2.0–3.5 wide	Obclav., cylindrical, straight or very slightly curved smooth, hila unthickened	Rather pale oliv. 1–7 transverse	25–85 × 3.5–4.5
P. tephrosiae	27.6 μm in diam., pseudoparench., dark oliv.	In small (loose) to large (dense) fascicles, smooth, suberect, somewhat flexuous, some geniculate, rarely branched, septate (up to 3) denticles with thickened scars	Light to mid oliv.	16.0–59.8 × 3.5–6.9	Cylindrical, to obclavatocylindrical smooth hila thickened	Light oliv. 2–6 transverse	20.7–64.4 × 3.5–4.6

conidia simplicia, solitaria, sicca, acropleurogena, pallide olivacea, usque 8 transverse septata, obclavata usque subcylindrica ad bases obconicotruncata vel subtruncata, ad apices subacuta usque obtusa hilo non incressato donata, glabra, 27.6–59.8 × 2.8–4.6 μm.

Infection spots amphigenous, very small to considerably large, necrotic, irregular, greyish-brown, sometimes surrounded by green haloes; colonies amphiphyllous, confined to the necrotic region of the spots, greyish-brown; hyphae immersed, septate, smooth, branched; stromata well-developed, irregular, pseudoparenchymatous, mid-olivaceous, 9.2–23.0 μm in diameter; conidiophores macronematous, mononematous, caespitose, densely packed, up to 3-septate, branched, suberect, slightly flexuous towards the apices, light-olivaceous, 11.5–59.8 × 3.5–4.6 μm; conidiogenous cells integrated, terminal, polyblastic, sympodial, denticulate, with short broad to slightly pointed denticles, sometimes denticles closely set; conidia simple, solitary, dry, acropleurogenous, light-olivaceous, up to 8-transversely-septate, obclavate to subcylindric, bases obconicotruncate to subtruncate, apices subacute to obtuse, hila unthickened, smooth, 27.6–59.8 × 2.8–4.6 μm (figure 2).

On living leaves of *Tinospora cordifolia* (Willd.) Miers. (Menispermaceae); October, 1979; Nichlaul (North Gorakhpur Forest Division); leg. A. N. Rai, KR 324, type IMI 243041.

The present fungus bears some resemblance of *P. cocculi* (H. Syd.) Deighton, one of the two species that have been reported earlier on the host family[1].

The length of conidiophores is more or less the same in both species. However, the author's collection differs from *P. cocculi* in other characters such as well-developed stroma; denticulate and narrower conidiophores; smooth, much shorter and narrower conidia with a small number of septa in the former, compared to less-developed stroma; nondenticulate and wider conidiophores; smooth, wrinkled or verruculose, much longer and wider conidia with a larger number of septa in *P. cocculi*. Hence, the new collection is described as a new species, *P. tinosporae*. So far no species of *Pseudocercospora* has been reported on the host genus.

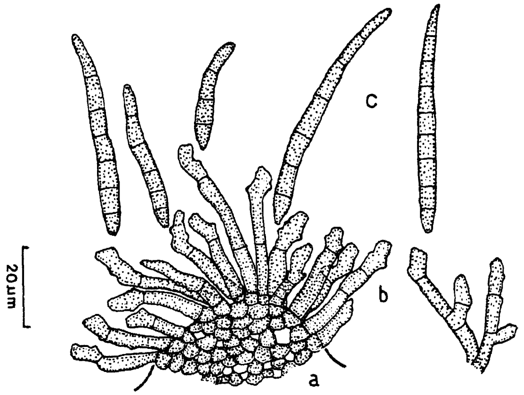

Figure 2. *Pseudocercospora tinosporae* A. N. Rai et Kamal sp. nov. **a,** Stroma; **b,** conidiophores; **c,** conidia.

The authors are grateful to the Director, CMI, Kew, England, for identifying the fungus.

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FIRST REPORT OF OCCURRENCE OF OOSPORES OF *PSEUDOPERONOSPORA CUBENSIS* ON TWO CUCURBITACEOUS HOSTS

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DOWNY mildew of muskmelon (*Cucumis melo* L.) and other cucurbitaceous plants, caused by *Pseudoperonospora cubensis* (Berk and Curt.) Rostow, is a serious disease that occurs annually under favourable climatic conditions. Under Punjab conditions, the pathogen is known to perpetuate in the form of active mycelium on self-sown or cultivated sponge gourd (*Luffa aegyptiaca* Mill.) vines growing in sheltered places during severe winter and also in open spaces during milder winters[1]. Bains *et al.*[2] also reported the occurrence of oosporic stage on a wild host *Melothria maderaspatana*. The present communication reports the occurrence of oosporic stage of *P. cubensis* on two cucurbitaceous hosts, pumpkin (*Cucurbita moschata* Duchesne) and sponge gourd (*Luffa aegyptiaca* Mill.) under local conditions.

Infected leaf samples showing typical circular to angular lesions were collected from off-season vines growing in the farm of the Department of Plant Pathology during the beginning of December, 1988. Microscopic examination of the infected tissue revealed the presence of oospores, which were smooth, globose in shape, yellow to light-brown, and with a thick outer wall. These were observed in good numbers and averaged 25 × 23 μm (figure 1c). The oospores were typical of *P. cubensis* of the type found on leaves of cucumber in China[3] and on leaves of parwal (*Trichosanthes dioica* Roxb.) in India[4], but slightly bigger in size than the ones reported by Bains *et al.*[2] on leaves of *M. maderaspatana.* We could not search for oospores from downy mildew-infected vines of various cucurbitaceous hosts during the regular growing season of the crop. To confirm the identity of the oospores, the infected samples were incubated at 20°C in a humid atmosphere. The typical dichotomously branched sporangiophores (figure 1a) with terminal papillate sporangia were observed. The sporangia were 25.0–37.5 × 12.5–25.0 μm in size (figure 1b). These observations confirm that the fungus was typical *P. cubensis.*

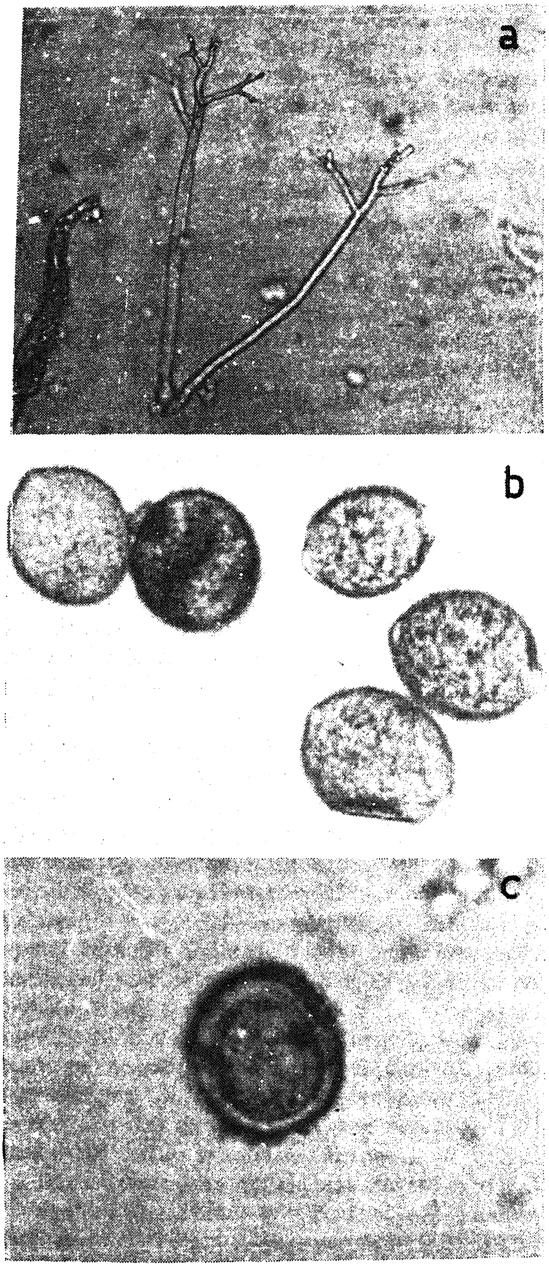

Figure 1a–c. **a,** A typical dichotomously branched sporangiophore of *Pseudoperonospora cubensis*; **b,** sporangia; **c,** a thick-walled oospore.

This report provides information on the possible mode of perpetuation of *P. cubensis* as oospores under a certain set of conditions which ensures the colonization of the same zone of the host tissue by mycelia of opposite sexual compatibility. It also shows that oospores, in addition to the vegetative stage, may be playing a role as a primary source of infection in the following year, particularly when self-sown vines/main season vines are available for colonization by the pathogen during winter.

The infected samples have been deposited in the Herbarium, Department of Plant Pathology, PAU, Ludhiana.

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POLYPHENOLS OF SOME INDIAN VEGETABLES

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VEGETABLES have assumed greater importance in human nutrition because of the recognition of the beneficial effects of dietary fibre and polyphenols, two constituents that were considered insignificant in the past. Dietary fibre includes all the unassimilable structural plant materials like cellulose, hemicelluloses and pectins. These materials have important effects on gut function because of their bulk, ability to absorb and retain water, and their being substrates for the bacteria of the gut[1]. The interest in food phenolics owes its origin to 'vitamin P', a group of polyphenols better known as 'permeability factors'. These compounds increase capillary resistance and thus prevent subcutaneous capillary bleeding. Rutin, the 3-rutinoside of quercetin, and flavonones of the *Citrus* fruits formed the principal components of vitamin P. In addition to their role in decreasing capillary bleeding, these compounds are reported to prolong life in scorbutic guinea pigs and help in overcoming vascular purpurea. But none of these substances has been shown to have a true vitamin effect and the designation was dropped in 1950 on the recommendation of the American Society of Biological Chemists and the American Institute of Nutrition[2]. At present the term 'bioflavonoids' is used to denote all the flavonoids exhibiting some pharmacological activity.

Though minerals, vitamins and even dietary fibre of vegetables are known in great detail, no conscientious effort seemed to be made to understand the phenolic chemistry of vegetables. A thorough knowledge of the phenols and their possible role in digestive processes would help to ascertain the beneficial/toxic effects of these compounds. In the present work, 11 leafy vegetables and 5 fruit vegetables commonly used in India were analysed qualitatively for their flavonoids and related compounds. Since glycosidic flavonoids partly lose their sugar residues in the human body to yield the aglycones[3], an attempt was made to identify the aglycones from the acid-hydrolysed extracts of the plants.

All the vegetables were collected fresh from Baroda. The fresh plant materials were analysed using standard procedures[4–6]. Authentic samples were used to confirm the identity of the flavonoids.

The distribution of the various phenolics in 16 vegetables is presented in table 1. All the leafy vegetables contained various flavonoids in the leaves. Flavonols were widely distributed, being found in all of them except *Colocasia*. Most of the vegetables contained quercetin and/or kaempferol and their various methoxylated derivatives. The 6/8-hydroxylated flavonols gossypetin and quercetagetin were seen in *Moringa* leaves. In cabbage and *Hibiscus sabdariffa* flavonols were in traces. The leaves of *Colocasia* contained flavones (apigenin and luteolin) instead of flavonols. None of the plants screened contained glycoflavones. Proanthocyanidins were seen in the leaves of *Moringa* while anthocyanins were located in *H. sabdariffa* and *Ipomoea aquatica*. Coumarins were present in both the umbelliferous plants screened, viz. *Anethum* and *Coriandrum*.

Proanthocyanidins were widespread among the fruit vegetables. Only *Cyamopsis tetragonoloba* was free of them. *C. tetragonoloba* was also peculiar in containing quercetin and kaempferol. Flavonols were in traces in both *Hibiscus esculentus* and *Vigna anguiculata*.

The presence of flavonols in most of the vegetables that are regularly consumed is noteworthy. It is also significant that most of the flavonoids possess 3′,4′-dihydroxy/dimethoxy substituents. Though their pharmacological effects have not been proved conclusively, the flavonoids are found to exert beneficial effects in more than fifty diseases. According to De Eds[7] the flavonoids with free hydroxyl groups at the 3′,4′-positions (quercetin, gossypetin, quercetagetin and luteolin) exert beneficial effects on the capillaries by (i) chelating metals and thus sparing ascorbate from oxidation, (ii) prolonging epinephrine action by inhibiting *O*-methyl transferase, and (iii) stimulating the pituitary–adrenal axis. The flavonoids with multiple methoxy groups (7,4′-diOMe kaempferol, 3′,4′-diOMe quercetin, etc.) play an important role in the circulatory system by reducing aggregation of erythrocytes (which occurs during illness or injury) by site-specific membrane surface effects, and improve the microcirculation within the body[8].

Since the coumarins are also known to act as diuretics, vasodilators and oestrogens[9], the vegetables containing them, viz. *Coriandrum* and *Anethum*, may exhibit these medicinal properties.

Since the flavonoids and/or coumarins are present in most of the vegetables, a critical appraisal of their

Table 1 *Polyphenols in some vegetables of India*

	Polyphenol*													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Leafy vegetables														
Brassica oleracea var. *capitata* L.						+								
Raphanus sativus L.			+	+	+									
Hibiscus subdariffa L.						+							+	
Moringa oleifera Lam.							+			+	+	+		
Trigonella foenum-graecum L.			+				+							
Sesbania sesban (L.) Merr.			+			+							+	
Anethum graveolens L.				+					+					+
Coriandrum sativum L.						+	+		+					+
Ipomoea aquatica Forsk.							+	+					+	
Argyreia nervosa Boj.						+								
Colocasia antiquorum Schott.	+	+											+	
Fruit vegetables														
Hibiscus esculentus L.									+			+		
Moringa oleifera Lam.												+		
Vigna unguiculata (L.) Walp.									+			+		
Cyamopsis tetragonoloba Taub.			+			+								
Dolichos lablab L.												+		

*1, Apigenin; 2, 3′,4′-diOMe luteolin; 3, kaempferol; 4, 4′-OMe kaempferol; 5, 7,4′-diOMe kaempferol; 6, quercetin; 7, 3′-OMe quercetin; 8, 4′-OMe quercetin; 9, 3′,4′-diOMe quercetin; 10, gossypetin; 11, quercetagetin; 12, proanthocyanidins; 13, anthocyanins; 14, coumarins.

metabolic fate and toxic properties, if any, is necessary. Simple phenols undergo various hydroxylation/dehydroxylation reactions or get oxidized to phenolic acids. Ingested quercetin has been found to get excreted as glucuronides or sulphates or to get metabolized to phenylacetic acids[10]. Phenylacetic acid is converted to phenylalanine or conjugated with glutamine to form phenylacetylglutamine and excreted in urine. Phenylacetic acid possesses antispasmodic properties[11].

The presence of proanthocyanidins in most of the fruit vegetables is interesting. These compounds undergo hydrolytic and polymerization reactions in the highly acidic alimentary tract and produce condensed tannins. The tannins are known astringents and are antimicrobial in nature. But they also interfere with the absorption of iron in the human body and may bind to enzymes, rendering them inactive.

The present work emphasizes the necessity of evaluating the various effects of phenolics in the human systam. It also brings to focus the importance of minor components of foods, which are often overlooked. A proper understanding of their role in the human body will facilitate the judicious use of vegetables effectively.

16 January 1989

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INHIBITION OF PHOTOSYNTHESIS BY OXYFLUORFEN

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OXYFLUORFEN, a diphenyl ether (DPE) group herbicide, has been found to be very effective against broad- and micro-leaved weeds[1,2]. The mechanism of herbicidal injury induced by oxyfluorfen is still unclear. Most of the DPE herbicides have a light-dependent mode of action but some do not[3,4]. Oxyfluorfen requires light for herbicidal action[4,5] and the evidence suggests that the photo-oxidative damage caused by oxyfluorfen involves photosynthetic electron transport[6-8]. Data in support of oxyfluorfen action independent of photosynthetic electron transport are also available[9-11]. Recently, Haworth and Hess[11] have shown that oxyfluorfen-induced herbicidal injury by generation of singlet oxygen is independent of photosystem I (PS I) electron transport, in agreement with the previous findings on the requirement of oxygen for DPE herbicidal activity[12,13].

In the present communication, we report that photo-oxidative damage to chloroplast membranes is due to the inhibition of photosystem II (PS II) electron transport by oxyfluorfen, which may involve binding of oxyfluorfen to pigment complex(es) or any other component of the membrane.

Seeds of *Oryza sativa* (var. CSR-4) obtained from the College of Agriculture, Indore, were soaked in water for 24 h and grown under laboratory conditions in plastic containers. Oxyfluorfen (1, 3, 5 and 7 ppm) was sprayed on 15-day-old rice plants, and studies were carried out every alternate day.

Chloroplasts were isolated from freshly cut leaves of rice plants according to Karabourniotis *et al.*[14] in ice-cold isolation medium containing 400 mM sucrose, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$, 10 mM KCl, 150 mM NH_4Cl, 2 mg/ml BSA (fraction V) and 4 g/l polyvinylpyrrolidone (PVP-10). The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged for one min at 3000 *g*. The supernatant was discarded and the pellet was suspended in minimal volume of isolation medium, but without PVP.

Chlorophylls were extracted in cold 80% acetone under dim green light and the amount of chlorophylls in the extract was estimated according to Mackenny[16].

Heat treatment of spinach chloroplasts (to destroy water splitting complex) was carried out by incubating chloroplasts at 48°C for 4 min and thereby stopping electron flow to PS II. Diphenylcarbazide (DPC) was used (final concentration 0.5 mM) as donor of electrons on oxidizing side of PS II.

Type C spinach chloroplasts were used in experiments to study the mode of action of oxyfluorfen and to calculate I_{50} values at different chlorophyll concentrations. For other experiments type C rice chloroplasts were used.

The photoreduction of 2,6-dichlorophenol indophenol (DCIP) (PS II activity, $H_2O \rightarrow DCIP$) was measured spectrophotometrically (Shimadzu model UV–VIS 160) according to Mohanty *et al.*[17] The chloroplasts were illuminated for 30 sec with saturating white light (50 W/m^2). The incident beam was passed through a water filter to cut off the infrared radiation. The reaction mixture in a final volume of 3 ml contained chloroplasts equivalent to 10 μg chlorophyll/ml, 20 μM DCIP, 20 mM Tris–Cl buffer (pH 7.4), 5 mM $MgCl_2$ and 5 mM NH_4Cl. The photoreduction of DCIP was measured spectrophotometrically at 605 nm and the rate of DCIP reduction was calculated using an extinction coefficient of 21 mM^{-1}.

Spraying of oxyfluorfen resulted in visible chlorosis, curling of leaf and appearance of irregular burnt patches. The visible injury was apparently more at higher concentrations (5 to 7 ppm) of oxyfluorfen. The effect of different concentrations of oxyfluorfen on total chlorophyll content of rice leaves is shown in figure 1. At low concentration of oxyfluorfen (1 ppm), there was a slight decrease in chlorophyll content up to 24 h after spray, followed by recovery. However, at higher concentrations of oxyfluorfen, there was no recovery but a further decrease. There was about 80% decrease in total chlorophyll content of leaves of rice plants sprayed

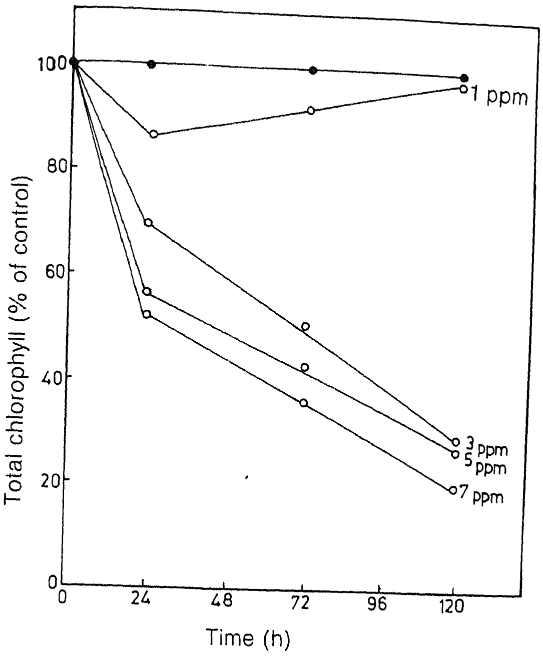

Figure 1. Effect of oxyfluorfen on total chlorophyll content (○) of rice plants (100% (control, ●) is equal to 1.734 mg total chlorophyll per/g fresh wt).

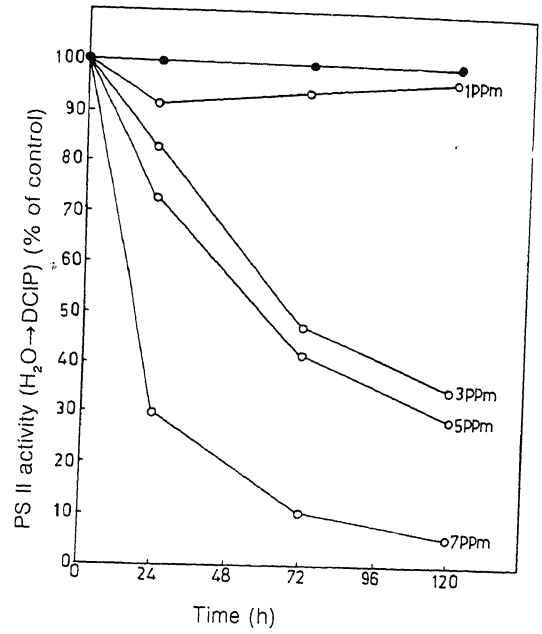

Figure 2. Effect of oxyfluorfen on PS II activity (○) of rice plants (100% (control, ●) is equal to 94.8 μmol DCIP reduced/mg chl/h).

with 7 ppm of oxyfluorfen 5 days after spray.

Oxyfluorfen also inhibited photosynthesis. Chloroplasts isolated from sprayed leaves showed inhibition of DCIP Hill reaction (figure 2). There was continuous decline in the rate of DCIP Hill activity of chloroplasts isolated from plants sprayed with 3, 5 and 7 ppm of oxyfluorfen but little and temporary inhibition by oxyfluorfen at low concentration (1 ppm). DCIP reduction 5 days after spray was inhibited by about 90% in chloroplasts isolated from rice leaves treated with 7 ppm oxyfluorfen.

In order to understand the mode of action of oxyfluorfen, isolated spinach chloroplasts were incubated with different concentrations (0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 ppm) of oxyfluorfen for one min and the rate of DCIP reduction was measured (figure 3). The I_{50} values for inhibition of DCIP reduction increased with increase in chloroplast concentration. The I_{50} value for 10 μg chl/ml was 0.44 ppm while for 30 μg chl/ml I_{50} was 0.72 ppm (inset of figure 3). The inhibition of PS II activity by oxyfluorfen was not reversed by the addition of DPC (table 1).

These results suggest that oxyfluorfen causes photo-oxidative damage to chloroplast membrane by inhibiting PS II activity and that oxyfluorfen inhibits electron transport chain beyond the DPC

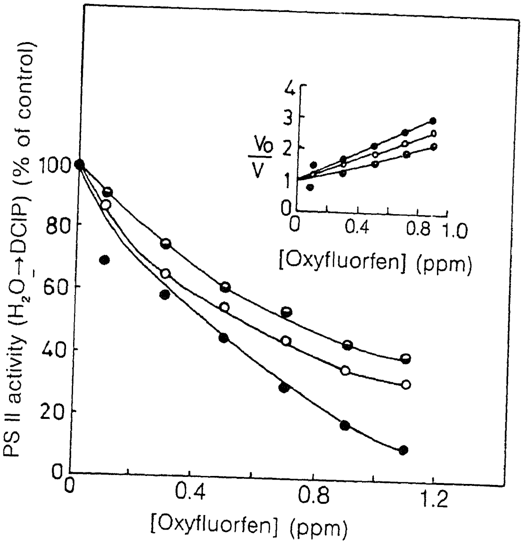

Figure 3. Effect of oxyfluorfen on PS II activity of isolated spinach chloroplasts (100% is equal to 390, 372 and 309 μmol DCIP reduced/mg chl/h by chloroplasts equivalent to 10 (○), 20 (●) and 30 (◒) μg chl/ml respectively). Inset shows a plot of V_0/V as a function of concentration of inhibitor. V_0 is rate of DCIP reduction in absence of inhibitor, V is rate in presence of inhibitor.

Table 1 *Effect of oxyfluorfen on PS II activity of normal and heat-inactivated spinach chloroplasts*

Treatment	PS II activity* (μmol DCIP reduced/mg chl/h) Control	Heat-treated
—	380 ± 67	48 ± 2
Oxyfluorfen	8 ± 1.2	0
Oxyfluorfen + DPC	4 ± 0.6	0
DPC	—	172 ± 3

*Each value is mean ± SD of triplicate samples. The data are representative as rate of DCIP reduction in control chloroplasts varied from 350 to 400 μmol DCIP reduced/mg chl/h in different preparations. Other conditions are as in text.

electron-accepting site on the oxidizing side of PS II. Increase in I_{50} with increase in chloroplast concentration suggests that oxyfluorfen may bind to pigment complex(es) or some other membrane component. The binding of oxyfluorfen affects PS II photochemistry[18].

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ELECTRICAL CONDUCTIVITY AS AN INDICATOR OF ORTHODOX AND RECALCITRANT SEED PHYSIOLOGY IN TROPICAL FOREST TREES

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THE majority of seeds used in agriculture show orthodox behaviour, that is to say, over a wide range of environmental conditions, their longevity may be increased in specific and predictable ways by decreasing temperature and moisture content. Recalcitrant seeds are those that do not obey these rules since they are killed when their moisture content is reduced below some relatively low value[1]. Such seeds cannot be preserved for more than a few months and some can only survive for a few weeks even when desiccation is prevented[2,3]. Diagnosis of the recalcitrant condition is not always straightforward, and there are many cases of seeds initially classified as recalcitrant and now known to be typically orthodox[4]. In the case of tropical forests where a number of forest tree species are recalcitrant, a quick and reliable method for identification of such species is absent.

Incorrect diagnosis of orthodox and recalcitrant seeds can affect decisions on methods of genetic conservation. The present study is the first attempt to find a measure for the identification of these two seed categories, taking seeds from truly recalcitrant and orthodox species. Experiments were conducted on freshly collected mature seeds of three truly recalcitrant species, viz. *Shorea robusta* Gaertn f., *Eugenia jambolana* Lam. and *Saraca indica* Linn., and four orthodox species, viz. *Bauhinia variegata* Linn., *Bombax malabarcium* D. C., *Dalbergia sissoo* Roxb. and *Melia azadirachta* Linn. Germination of seeds in the seven species was found to be 80 to 100%. Electrical conductivity was determined[5] hourly up to 24 h after immersing seeds in water at 20°C.

Figure 1 shows that electrical conductivity in recalcitrant seeds ranged from 1.36 to 14.72 $\mu s\,cm^{-1}\,g^{-1}$.

In the case of orthodox seeds the range was 27.84 to 171.56 μs cm^{-1} g^{-1} (figure 2). Since electrolyte leakage is an indicator of membrane disruption,[6] the results of the present study suggest that, in orthodox seeds, cell membranes lose their integrity because of drying to a greater extent on the tree itself[7,8]. The

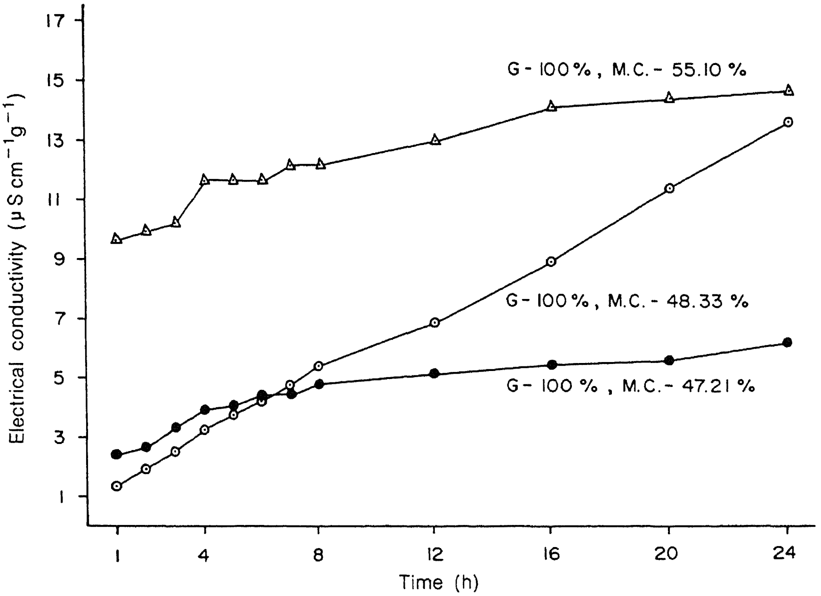

Figure 1. Time course of electrolyte leakage from recalcitrant seeds of *S. robusta* (○), *S. indica* (●), and *E. jambolana* (△) immersed in water (G, germination; MC, moisture content).

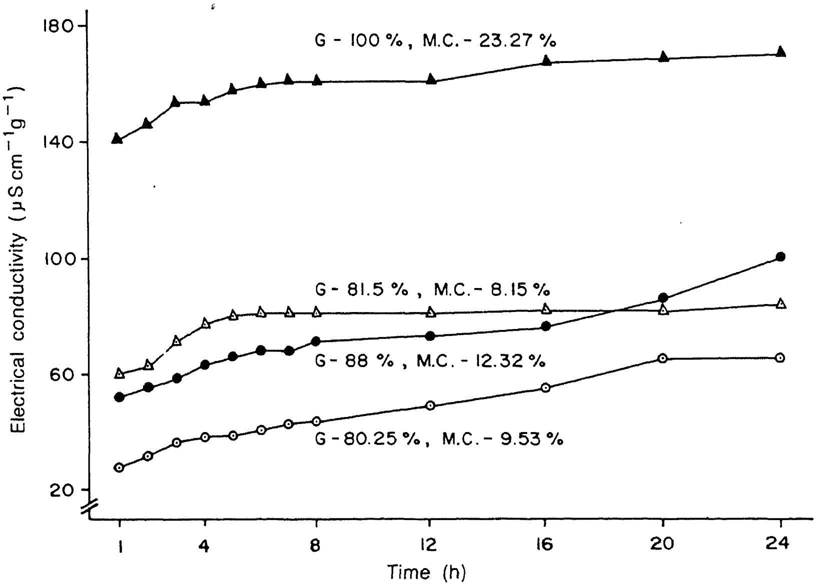

Figure 2. Time course of electrolyte leakage from orthodox seeds of *B. variegata* (○), *D. sissoo* (●), *B. malabaricum* (△), and *M. azadirachta* (▲) immersed in water (G, germination; MC, moisture content).

results can also be interpreted on the basis of low moisture content in the orthodox seeds, except *M. azadirachta*, where seed moisture content was comparatively higher (23.27%) owing to the fleshy outer covering, which delayed the process of seed desiccation on the tree itself. The higher seed moisture content in this species can also be explained on the basis that mature seeds were considered for the experiment without manipulating seed moisture. However, 20% germination has been reported in this species for seeds with 4% moisture content[4]. The high moisture content (48.33 to 55.10%) in recalcitrant seeds indicates that, because of slow desiccation rate, membrane integrity is maintained and leakage is less. This situation may have evolved because orthodox species benefit from desiccation while slow desiccation rate is protective in recalcitrant species[9]. In contrast to the higher electrolyte leakage from orthodox seeds and less leakage from recalcitrant seeds, the pattern of rate of loss of electrolyte was similar in both the categories (tables 1 and 2). Maximum electrolyte leakage was observed during the first hour of soaking, followed by a decline. This decline and/or stabilization in the rate of loss can be attributed to the re-establishment of membrane integrity in the seeds due to a repair mechanism on hydration.

Among the seven species in the present study, *M. azadirachta* was earlier recognized as recalcitrant[10] but later included in the orthodox category owing to the presence of structural features such as a fleshy outer covering on a relatively thin-walled stone which contains the kernel. However, if kernels are removed from dried stones before the germination test, seeds germinate perfectly satisfactorily. It is not clear in this case whether the stones prevent germination entirely or delay it considerably[4]. Higher electrolyte leakage observed in this study from seeds of this species also confirms their orthodox nature.

Table 1 *Rate of loss of electrolytes from recalcitrant seeds of three forest tree species after different soaking periods*

Soaking period (h)	Electrolyte loss rate (%)		
	S. robusta	*S. indica*	*E. jambolana*
1	9.96	37.67	65.37
2	4.24	4.46	1.95
3	4.49	10.01	1.90
4	4.98	10.81	9.65
5	3.94	2.23	0
6	3.46	4.29	0
7	3.88	2.23	3.80
8	4.76	4.29	0.0
12	18.17	6.51	5.78
16	7.91	4.46	8.74
20	18.3	2.07	1.90
24	15.83	9.97	1.91

Table 2 *Rate of loss of electrolytes from orthodox seeds of four forest tree species after different soaking periods*

Soaking period (h)	Electrolyte loss rate (%)			
	A. indica	*D. sissoo*	*B. variegata*	*S. malabaricum*
1	82.19	51.60	42.29	70.93
2	3.42	3.23	5.77	3.42
3	4.10	3.22	7.68	10.25
4	0	4.83	1.92	6.84
5	2.73	3.22	0	3.42
6	0.68	1.61	3.84	0.84
7	0.68	0	3.84	0
8	0	3.22	1.92	0
12	0	1.61	7.68	0
16	4.10	3.23	9.61	0.86
20	0.68	9.67	15.39	0
24	1.36	14.51	0	3.42

Electrical conductivity of seeds could be used as a measure for identification of orthodox ($>20\ \mu S\ cm^{-1}\ g^{-1}$) and recalcitrant seeds ($<20\ \mu S\ cm^{-1} g^{-1}$). It is further suggested that conductivity measurements should be performed on freshly collected seeds to avoid desiccation effects during storage.

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PHYSIOLOGY OF MUTANT STRAINS OF *ERWINIA CAROTOVORA* PRODUCING L-ASPARAGINASE

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SINCE the first demonstration[1] of L-asparaginase (L-asparagine amidohydrolase EC 3.5.1.1) as an anticancer enzyme, production of the enzyme by a variety of microbes has been investigated in many laboratories. The various sources reported, such as *Pseudomonas fluorescens*[2], *Serratia marcescens*[3], *Escherichia coli*[4], *Erwinia carotovora*[5] and *Proteus vulgaris*[6], provided active and immunologically different enzyme preparations. All these species were evaluated in the author's laboratory. As *E. carotovora* was shown to be a promising culture, it was mutated by UV and gamma radiation and by treatment with chemical mutagens as part of a strain improvement programme. The mutant strains derived by these traditional methods were further investigated by a classical method of genetic recombination reported by Barnes *et al.*[7]

The original wild-type strain *E. carotovora* was a gift from Prof. Wade of Microbiological Research Establishment (UK). It was mutated by the routine methods such as UV and gamma radiation and treatment with chemical mutagens. The mutants were screened and stock cultures were maintained. Working cultures were obtained by streaking the stock culture and transferring isolated colonies on tryptone yeast (TY) agar slants (in g/l: Tryptone, 5.0; yeast extract, 2.0; lactose, 5.0; K_2HPO_4, 1.0; NaCl, 5.0; agar, 15.0; pH 6.8–7.0). Purity was checked periodically.

Auxotrophic strains were isolated on minimal agar (in g/l: NaCl, 0.2; Na_2SO_4, 2.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; lactose, 10; pH 7.0) supplemented with individual amino acids. To determine antibiotic resistance, the following antibiotics were added to minimal agar: penicillin, 200–2000 units/ml; ampicillin, 10–20 μg/ml; erythromycin, 5–10 μg/ml; chlortetracycline, 10–100 μg/ml; chloramphenicol, 250–500 μg/ml; and streptomycin, 10–20 μg/ml.

Complete medium (TGY) was used for the fermentation process (in g/l: Tryptone, 20.0; yeast exrtact, 5.0; monosodium glutamate, 15.0; lactose, 10.0; K_2HPO_4, 1.0; NaCl, 1.0; $CaCO_3$, 5.0; $MgSO_4 \cdot 7H_2O$, 0.2; pH 7.4).

The depleted mutant strains of *E. carotovora* were treated with a mixture of amino acids which included monosodium glutamate, L-asparagine, tryptophan, methionine, alanine, serine and threonine. After eliminating one of these amino acids from the mixture, their polyauxotrophic character was determined. They were screened to select producer and non-producer strains and tested for growth in presence of antibiotics. The producer strains were resistant particularly to streptomycin and chlortetracycline antibiotics at the higher concentration. They required specifically monosodium glutamate as carbon source. The non-producer strains lacked this requirement and their growth was inhibited at lower concentrations of streptomycin and chlortetracycline. All the strains—wild and polyauxotrophic—were however sensitive to penicillin and ampicillin.

The mutants were mated according to the procedure of Barnes *et al.*[7] Donor, recipient and recombinant cells were characterized by observing their growth on the third day. Only genetic recombinants grew and were picked up as isolated colonies. They were subcultured five times on minimal agar plates and stock cultures were prepared. After purification they were transferred to agar slants, incubated for 16 h, and inoculated into production flasks directly for fermentation studies. The fermentation was carried out in 100 ml medium in a 500 ml Erlenmeyer flask. The cultures were incubated for 24 h at 37°C on a rotary shaker (250 rpm). Changes in fermentation parameters in the recombinants were considered as evidence of successful application of the technique adopted. L-Asparaginase activity, being intracellular, was assayed by the routine Nesslerization method[8]. One IU represents 1 μmol of ammonia liberated per min at 37°C in 0.05 M borate buffer, pH 8.5.

In table 1, the polyauxotrophic and the streptomycin-sensitive (Sm^S) strains are shown as non-producers while the streptomycin-resistant (Sm^R)

Table 1 *L-Asparaginase production by mutant strains of E. carotovora*

Mutant strain	Phenotypic characters	PCV* (%)	Enzyme activity (IU/ml)
389	MSG$^+$ Ala$^+$ Sm^R	3	0.2
189	Asn* Gln$^+$ Ala$^+$ Sm^S	4	0.08
213	MSG$^+$ Tr$^+$ Met$^+$ Sm^R	3	0.38
56	Asn$^+$ Tr$^+$ Met$^+$ Sm^S	5	0.0
106	Asn$^+$ Met$^+$ Tr$^+$ Sm^S	3	0.0
122	MSG$^+$ Gln$^+$ Sm^R	5	1.5

*Packed cell volume.

Table 2 *L-Asparaginase production by recombinants of E. carotovera obtained by genetic recombination*

Recombinant	Mutant strains in cross	PCV (%)	Enzyme activity (IU/ml)
R1	389 × 213	3.0	1.58
R2	189 × 56	3.0	1.43
R3	106 × 213	2.5	3.13
R4	106 × 122	2.5	3.13
R5	122 × 213	2.5	5.93
R6	389 × 122	2.0	6.10
R7	389 × 106	3.0	2.10
R8	56 × 109	3.0	1.30

strains are seen as poor producers. These strains, when hybridized, yielded, among others, the recombinants R5 and R6, which were at least four times more active than the original strains (table 2). It was further observed that the mutant strains requiring monosodium glutamate specifically and showing streptomycin resistance produced these high yielding recombinants. Hence Sm^R was considered a suitable selective marker. The prototrophic recombinants were also found to be induced when L-asparagine, at 0.1–0.3% was added to the culture medium; there was a 3.5-fold increase in enzyme activity. The parental strains were never capable of showing such induction.

The author thanks Prof. Wade of Microbiological Research Establishment, UK, for his kind gift of *Erwinia carotovora*; Dr A. Subrahmanyam and Mr Vijay K. Singh for making available the mutants and for their maintenance; and Dr S. R. Naik and Dr J. G. Shewale, for encouragement and helpful discussions.

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EFFECT OF *RHIZOBIUM* IN ASSOCIATION WITH GRANULAR INSECTICIDES ON NODULATION AND YIELD IN SOYBEAN

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INSECTICIDES containing mercury, copper or zinc, when used for seed protection, were found to be extremely toxic to *Rhizobium* sp.[1–4] Oblisami *et al.*[5], found in *in vitro* tests that endrin, carbofuran and disulfoton inhibited the growth of *Rhizobium* from red gram (*Cajanus cajan*). Seed inoculation with *Rhizobium* is advocated for achieving higher grain production in soybean. We therefore studied the effect of pesticides on nodulation *vis-a-vis* yield of soybean to evolve an effective combination of pesticide and rhizobial culture.

The experiment was conducted in randomized block design at the Research Farm of IARI, New Delhi. Soybean seeds, var. Harosoy-63, were coated with effective culture of *Rhizobium japonicum* (IARI strain, obtained from the Division of Microbiology, IARI) using 10% sucrose solution before sowing. The inoculated seeds were air-dried. Granular insecticides phorate 10, quinalphos 5, mephosfolan 5 and carbofuran 3 were drilled in soil furrows before sowing. All the insecticides except phorate were applied at 3 g/m row and phorate was applied at 1.5 g/m row. There were six treatments, each replicated four times. The size of each plot was 3.0 × 2.25 m. Row-to-row and plant-to-plant distances were 37.5 and 5 cm respectively.

The crop was given two light irrigations before uprooting, one at the beginning of flowering and the other at early pod formation stage, in addition to normal rainfall received during the crop season. Ten plants were uprooted from each plot each time.

Table 1 *Effect of Rhizobium inoculation on nodulation, height of plants and grain yield of soybean with and without insecticides*

	Mean number of nodules*		Mean weight of nodules (g)*				Mean height of plants* (cm)		Grain yield	
			Fresh		Dry					
Treatment	30 DAS	48 DAS	30 DAS	48 DAS	30 DAS	48 DAS	30 DAS	48 DAS	Per plot (g) mean	Per ha. (Q)
Control	0	0.25	0	0	0	0	227.50	288.25	646.06	9.57
Rhizobium alone	186.50	280.25	1.0965	2.63	0.30	0.94	242.00	395.25	771.42	11.42
Rhizobium + phorate	196.25	319.50	1.3475	3.54	0.35	1.233	243.25	370.75	1137.85	16.85
Rhizobium + quinalphos	201.50	322.00	1.5420	3.39	0.41	1.197	257.25	404.50	983.56	14.57
Rhizobium + mephosfolan	183.25	303.25	1.4586	2.86	0.34	1.021	243.50	375.50	858.21	12.71
Rhizobium + carbofuran	184.75	293.75	1.4757	3.43	0.39	1.330	242.50	385.25	858.21	12.71
SE of mean	19.73	21.34	0.1272	0.24	0.036	0.108	4.94	27.48	96.61	
C.D. at 0.05	59.45	64.31	0.3840	0.72	0.108	0.326	14.88	N.S.	291.15	
C.V. (%)									22.7	

DAS, Days after sowing. *Mean of 10 plants.

Nodules were removed carefully from each plant and cleaned and counted. Fresh weight of the nodules (ten plants in each treatment) was determined on an electrical microbalance. Afterwards, the nodules were dried at 80°C for three days before recording their dry weight. The height of each plant was measured from soil level to the tip of the stem. At harvest, grain yield per plot was recorded and analysed. The yield per plot was converted into yield per hectare.

The data are shown in table 1. In the uninoculated plants there was virtually no nodulation in pre-flowering or early pod formation stage of the crop. There were no significant differences in nodule number between treatments with *Rhizobium* alone and with *Rhizobium* in association with insecticides. The maximum number of nodules was recorded with *Rhizobium* in association with quinalphos. The results agree with those reported by Mundade *et al.*[6] who observed increased nodulation in peanut (*Arachis hypogea*) treated with *Rhizobium* association with carbofuran.

A significant increase in nodule fresh weight was recorded with *Rhizobium* + quinalphos at the pre-flowering stage and with *Rhizobium* + phorate at early pod formation stage.

Rhizobium in association with insecticides had the beneficial effect of increasing plant height at pre-flowering stage. A significant increase in height was however, recorded only when *Rhizobium* was in association with phorate, quinalphos or mephosfolan insecticides. At early pod formation stage, no significant difference in height was observed.

All these findings indicate the effectiveness of the combination of *Rhizobium* culture and insecticides.

Rhizobium inoculated seeds recorded an increase of 19.4% yield over uninoculated seeds but significantly higher yield (43.20%) was recorded when *Rhizobium*-inoculated seeds were sown in association with phorate. The reason for this may be significantly greater number of nodules as well as higher nodule fresh and dry weight at early pod formation stage. These observations reveal that seeds inoculated with *Rhizobium* culture can be safely sown in association with these granular insecticides, preferably with phorate-10.

Thanks are due to Dr V. R. Balasundaram, Division of Microbiology, IARI, New Delhi, for supply of IARI strain of *Rhizobium japonicum* culture.

12 January 1989; Revised 1 March 1989

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AZOTOBACTER CHROOCOCCUM IN ROOT, STEM AND LEAF TISSUES OF CELLS OF *TRITICUM AESTIVUM* L. AND *TRITICUM DURUM* L.

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NITROGEN fixation by free-living bacteria associated with plants had received a good deal of attention[1]. It is known that efficiency of nitrogen fixation is more common in symbiotic associations of bacteria and plants but Parker[2] suggested that it should not be confined to root nodules and that other existing associations for nitrogen fixation must not be ignored. In this context the intracellular detection of *Azotobacter chroococcum*, a free-living nitrogen fixer, in root, stem and leaf tissues of *Triticum aestivum* L. and *Triticum durum* L. and its probable role in the nitrogen economy of wheat are presented.

Wheat genotypes of RAJ-1555 and Kiran of *T. durum* L., and APAU-1577 and DWR-16 of *T. aestivum* L. were obtained from Wheat Breeder, AICRP on Wheat, Dharwad. They were surface-sterilized following the method of Bhide and Purandare[3] and grown aseptically on Trelease and Trelease agar media[4] in 2 l conical flasks. After 10 days of growth, transverse sections of roots, stem and leaves were observed under a phase contrast microscope.

At higher magnifications parenchymatous cells of the root cortex (figure 1a), the parenchymatous cells of the leaf sheath, especially those near the peripheral region of the stem (figure 1b), and the

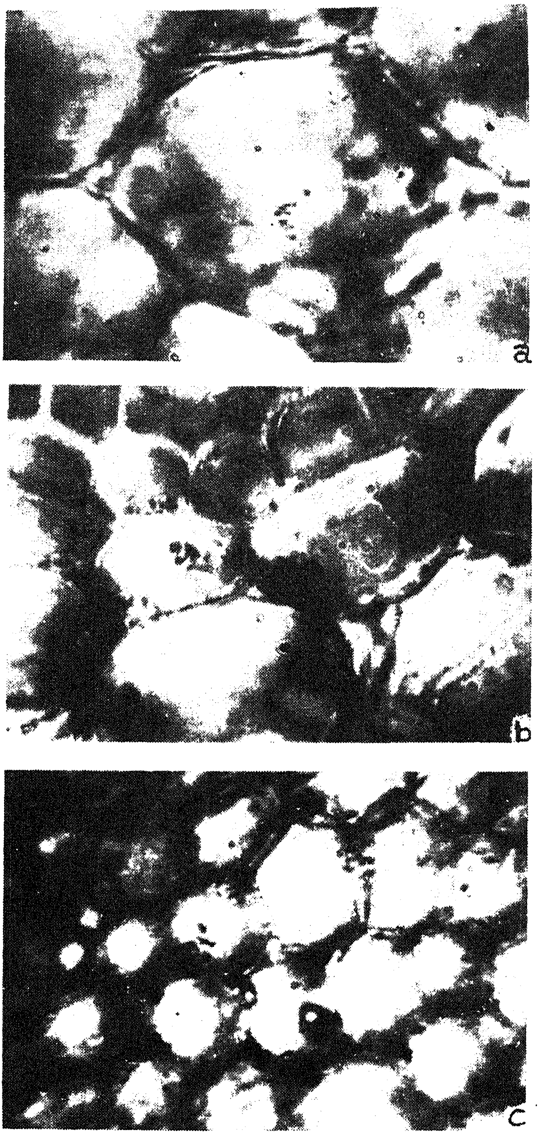

Figure 1a–c. Photomicrographs of T. S. of (**a**) roots (× 1500), (**b**) stem (× 900) and (**c**) leaves (× 675) of *Triticum* showing *Azotobacter chroococcum* within the cells of the tissues.

mesophyll region of leaf (figure 1c) were found to be teeming with microbodies, both single and pairs. Movement was arrested by adding 75% ethanol. Morphologically the microbes appeared coccoidal.

The presence of bacteria inside the above tissues was confirmed by placing aseptically prepared sections on nitrogen-free Norris glucose agar. At 30°C brown-pigmented bacteria appeared around leaf, stem and root sections. The colonies were raised, smooth, cream-white initially, mucoid, and on prolonged incubation formed pigment. They were capsulated, nonsporulating, aerobic, motile and gram-negative. The isolates could utilize glucose, mannitol, sucrose and starch but not rhamnose. Following Bergey's Manual[5], the isolates have been identified as a variant of *Azotobacter chroococcum.* Pure cultures obtained from the host tissue fixed nitrogen (2.9 to 3.8 mg N/g of glucose as estimated by the micro-Kjeldahl method). It remains to be investigated whether nitrogen fixation occurs at the sites where the bacteria were localized, and if so, its intensity and benefit to the host plant. These results and those of others[6,7] support the presence of *Azotobacter* in the cortical cells of the host.

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ANTIBACTERIAL ACTIVITY OF A POLYCATION

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POLYCATIONS are linear and contain aromatic, aliphatic and quaternary salt units. The simpler ionone polymers are structurally related to spermine, spermidine and their methylated derivatives[1]. Polymers containing cationic centres form very interesting polysalts, which have a variety of uses[2]. Since a polycation of high positive charge could lead to organic materials of high conductivity, this type of polymer is of considerable interest. The synthetic polycation polylysine inhibits the infectivity of tobacco mosaic virus as well as some animal viruses and bacteriophages, and the growth of numerous bacteria[3]. Polycations constitute an important group of biologically active molecules that have shown considerable promise in prophyl axis and chemotherapy of viral infections[4]. It is well known that polycations interact with nucleic acids and α-amylases[5]. This communication reports antibacterial activity of a synthetic polycation.

The polycation shown below was synthesized according to the procedure described earlier[6,7]. It was screened for activity against eight bacterial species, viz. *Escherichia coli*, *Pseudomonas alkaligenes*, *Bacillus subtilis*, *Proteus vulgaris*, *Serratia marcescens*, *Vibrio cholerae*, *Shigella sonnei* and *Salmonella typhi*. Antibacterial activity was assessed by the cup plate method[8]. The base layer medium was melted on a water bath and cooled to 55°C. Aliquots of 20 ml of liquid medium were distributed to each petri dish and allowed to solidify. The seed layer medium was melted and cooled to 45–50°C with gentle shaking. Overnight culture (2%) was added asceptically to the seed layer medium, which was then mixed thoroughly and quickly poured into petri dishes containing the base layer. After solidification and cooling, cups were made by punching into the set agar with a sterile cork borer and scooping out the punched part. The diameter of each cup was 10 mm. To these cups, 0.1 ml (9 μg) of a solution of the polycation in distilled water was added using a sterile. The plates were kept in the cold for an hour to facilitate diffusion. They were then incubated at 37°C for 48 h. Aqueous phenol (5%) was used as positive control. The results are summarized in table 1.

On the basis of these observations the polycation is highly active against *E. coli* and *P. alkaligenes*, moderately active against *S. typhi*, and less active against *P. vulgaris* and *S. marcescens*. It was inactive against the other microorganisms tested.

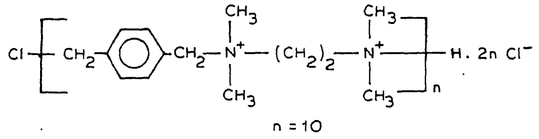

Table 1 *Antibacterial activity of polycation*

Organism	Zone of inhibition (mm)
E. coli	25–32
P. alkaligenes	25–32
S. typhi	20–25
P. vulgaris	15–20
S. marcescens	15–20
V. cholerae	—
S. sonnei	—
B. subtilis	—

—, No inhibition.

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PHOSPHAMIDON-INDUCED CHANGES IN HEPATIC ENZYMES OF MOUSE

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PHOSPHAMIDON (*O,O*-dimethyl-*O*-1-methyl-2-choloro-2-diethylcarbamoylvinyl phosphate) is an organophosphate systemic acaricide. It is extensively used against sucking, chewing and mining insects. The liver is the organ most significantly involved in the metabolism and storage of different pesticides. The phosphatases of the liver play an important role in the biochemical transformation of these substances. The present study was undertaken to evaluate the effects of phosphamidon on the activity of hepatic enzymes acid and alkaline phosphatase and glucose 6-phosphatase in mice.

Swiss albino male mice obtained from an inbred colony were treated with LD_{50} and half-LD_{50} dose of phosphamidon, i.e. 11.2 mg/kg body wt and 5.6 mg/kg body wt respectively, by gavage. Autopsies were done at 3, 6, 12 and 24 h, and 3, 7, 14, 21 and 28 days post-treatment. The liver was removed quickly and processed for biochemical estimations. Alkaline and acid phosphatases were estimated colorimetrically by the method suggested by Fiske and Subbarow[1] and glucose 6-phosphatase activity was determined by Swanson's method[2].

The results are shown in tables 1 and 2. The higher dose of phosphamidon caused significant increase in acid phosphatase activity. The lower dose did not cause significant change in acid phosphatase activity. The increase in hepatic acid phosphatase activity in treated animals may be due to destruction of lysosomal membranes in the cells, resulting in release of the enzyme[3]. Similar changes in hepatic acid phosphatase activity induced by other organophosphate pesticides have been reported by many workers[4-7].

A significant decrease in alkaline phosphatase activity was observed at the higher dose of phospha-

Table 1 *Effect of phosphamidon (11.2 mg/kg body wt) on hepatic acid and alkaline phosphatase and glucose 6-phosphatase activities in Swiss albino mice*

Time after treatment	Enzyme activity (mg Pi released/g/h)					
	Acid phosphatase		Alkaline phosphatase		Glucose 6-phosphatase	
	Control	Treated	Control	Treated	Control	Treated
3 h	2.07 ± 0.01	5.00 ± 0.40^y	3.12 ± 0.04	3.20 ± 0.01	13.53 ± 0.21	8.20 ± 0.35^z
6 h	2.07 ± 0.01	4.11 ± 0.37^y	3.12 ± 0.04	2.96 ± 0.07^y	13.53 ± 0.21	9.35 ± 0.22^z
12 h	2.07 ± 0.01	3.34 ± 0.31^y	3.12 ± 0.04	2.62 ± 0.01^z	13.53 ± 0.21	10.22 ± 0.17^z
24 h	2.07 ± 0.01	3.06 ± 0.21^y	3.12 ± 0.04	2.80 ± 0.10^z	13.53 ± 0.21	10.70 ± 0.08^z
3 days	1.97 ± 0.07	5.18 ± 0.17^z	3.22 ± 0.05	2.98 ± 0.02^y	13.96 ± 0.10	12.46 ± 0.26^x
7 days	2.08 ± 0.06	5.97 ± 0.31^z	3.20 ± 0.02	2.80 ± 0.03^y	13.26 ± 0.14	9.35 ± 0.17^z
14 days	2.08 ± 0.08	5.39 ± 0.09^z	3.08 ± 0.01	2.84 ± 0.05^y	13.97 ± 0.07	11.18 ± 0.17^z
21 days	2.13 ± 0.02	5.46 ± 0.09^z	3.20 ± 0.02	2.91 ± 0.03^y	12.45 ± 0.21	8.55 ± 0.16^z
28 days	1.99 ± 0.01	5.40 ± 0.18^z	3.11 ± 0.04	2.98 ± 0.01^x	13.31 ± 0.16	7.48 ± 0.18^z

Each value is the mean of 5 animals ±SE.
Significance of difference: $^xP < 0.05$, $^yP < 0.01$, $^zP < 0.001$.

Table 2 *Effect of phosphamidon (5.6 mg/kg body wt) on hepatic acid and alkaline phosphatase and glucose 6-phosphatase in Swiss albino mice*

Time	Enzyme activity (mg Pi released/g/h)					
	Acid phosphatase		Alkaline phosphatase		Glucose 6-phosphatase	
	Control	Treated	Control	Treated	Control	Treated
3 h	3.04 ± 0.03	3.69 ± 0.30	3.76 ± 0.19	3.69 ± 0.23	10.13 ± 0.15	9.32 ± 0.33
6 h	3.04 ± 0.03	3.58 ± 0.25	3.76 ± 0.19	4.20 ± 0.11	10.13 ± 0.15	9.51 ± 0.31
12 h	3.04 ± 0.03	3.10 ± 0.55	3.76 ± 0.19	4.00 ± 0.07	10.13 ± 0.15	9.11 ± 0.36
24 h	3.04 ± 0.03	3.56 ± 0.31	4.02 ± 0.47	6.79 ± 0.44^x	10.13 ± 0.15	8.34 ± 0.13^z
3 days	3.32 ± 0.48	2.23 ± 0.36	4.21 ± 0.38	5.20 ± 0.99^x	11.91 ± 0.15	7.20 ± 0.52^z
7 days	3.17 ± 0.25	2.63 ± 0.14	4.35 ± 0.48	4.75 ± 0.55	10.93 ± 0.67	6.89 ± 0.21^z
14 days	3.29 ± 0.49	2.14 ± 0.12	3.97 ± 0.36	4.40 ± 0.49	11.06 ± 0.36	10.23 ± 1.56^x
21 days	3.29 ± 0.47	2.43 ± 0.25	4.18 ± 0.10	4.40 ± 0.37	11.72 ± 0.80	10.40 ± 0.22
28 days	3.32 ± 0.54	2.72 ± 0.12	3.92 ± 0.03	3.99 ± 0.62	10.59 ± 0.69	9.70 ± 0.37^x

Each value is the mean of 5 animals ±SE.
Significance of difference: $^xP < 0.05$, $^zP < 0.001$.

midon, whereas the lower dose did not produce any significant change except at 24 h and 3 days after treatment. The decrease in alkaline phosphatase activity may be due to massive cell damage as suggested by Dinman[8] and also observed by the present authors[9]. Other organophosphate insecticides also act on this enzyme in a similar way[6,7]. Increase in the activity of alkaline phosphatase (24 h and 3 days) may be to counteract the effect of the toxic action of the insecticide, as also observed and suggested by other workers[4,5,10,11].

A significant decrease in glucose 6-phosphatase activity was observed after the treatment at both the doses. The decrease in activity of this enzyme in treated animals is an indication of cellular damage, which obstructs cellular metabolic activity under the toxic influence of phosphamidon[12]. Other organophosphate pesticides which cause pathological changes in hepatic tissue also interfere with the glucose 6-phosphatase activity[4,5].

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CURRENT SCIENCE—50 YEARS AGO

PROF. E. O. LAWRENCE*

ERNEST ORLANDO LAWRENCE, who has won the signal distinction of a Nobel Prize for Physics for 1939, began his research career at the University of Chicago in the year 1924, under Professor Swann, on the photo-electric effect in potassium vapour as a function of the frequency of light. With Prof. Swann, Lawrence moved to the University of Yale where he completed these investigations which formed the subject of a dissertation for his Ph.D. degree. In 1928 Lawrence migrated to the University of California at Berkeley and there he is now continuing his investigations which have brought him fame and the approbation of the scientific world. Much of Lawrence's earlier work was concerned with studies of photo-electric phenomena. It was in 1931 that Lawrence began to tackle the problem of production of high speed ions. R. Wideroe had by then suggested an apparently simple method of producing high voltage ions using only relatively low applied voltages, and had himself succeeded in obtaining $50{,}000^{\mathrm{v}}$ potassium ions in a tube to which a maximum voltage of half that value had been applied. Lawrence took up the idea and improved upon it with perseverence and ingenuity.

The principle of the method of Wideroe as at first developed by Lawrence is as follows: A series of cylindrical electrodes arranged along the length of an evacuated tube are attached alternately to either terminal of the inductance of a high frequency oscillatory circuit. A high frequency voltage applied in this manner, produces at any instant, electric fields of opposite direction and equal magnitude between successive electrodes. If at any one instant an ion finds itself between the first and second tubes it will be accelerated into the second tube, and if the time consumed in passing through the field-free space inside this tube is equal to the half period of the oscillator, the ion will arrive between the second and third tubes, with the field reversed in direction in such a manner that it will receive an additional acceleration while passing into the third tube. If the tubes are made successively longer to take account of the increasing velocity of the ion, for every frequency of applied oscillations there will be a corresponding voltage applied such as will cause the ion to move up through the series of tubes in synchronism with the oscillating field, gaining between each pair of tubes an increase in kinetic energy corresponding to the applied potential difference. Sloan and Lawrence showed that by the above method they could produce mercury ions of 1,260,000 volts using 30 accelerator brass tubes and a high frequency voltage of 42,000 on a wavelength of 30 meters.

The difficulty with the above type of apparatus is that the lengths of the accelerator tubes used towards the end of the path become very large even for heavy ions like Hg ion and the whole apparatus becomes inordinately long. To overcome this difficulty Lawrence conceived the idea of bending the path of the ions into circular orbits by a magnetic field and thus the first 'cyclotron' was born. In this device the electrodes in the form of semicircular hollow brass plates are mounted with their diametral edges adjacent in a vacuum and in a uniform magnetic field perpendicular to the plane of the plates. An oscillating electric field is produced by high frequency oscillations applied to the electrodes in the diametral region where ions are accelerated. They then describe semi-circles inside the electrodes, the time taken being arranged to be a half period of the oscillations. When they re-emerge into the diametral region they are again accelerated and then describe second semi-circles. Repetition of this process gives the ions very high velocities. The focussing action of the electric and magnetic fields gives narrow intense beams. Using a magnet with pole faces 11 inches diameter a narrow beam of current of 10^{-9} amperes consisting of protons of 1.22×10^{6} volts velocity has been obtained from a maximum applied voltage of 4,000.

In 1934, Lawrence and Livingston constructed an improved and larger apparatus of the above type, and attained a maximum speed of the hydrogen atoms corresponding to 5,000,000 electron volts the ionic current being 1/3 microampere.

Lawrence and Cooksey have recently described their latest cyclotron called also 'magnetic resonance accelerator'. The pole faces of the magnet are 3½″ apart and have a diameter of 27½″ although the

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actual effective diameter of the pole faces is 42″. The atomic beams can be led out into the air through thin platinum windows and their range amounts to several centimeters in air. A still larger cyclotron is now in the making.

When high speed deuteron streams are made to fall on berylium atoms, streams of neutrons are produced so powerful in their biological action that they are equivalent to the gamma radiations from 100 grams of radium. Accordingly, for the protection of the operator, the cyclotron is controlled from a distance of 40 feet from the apparatus with suitable intervening absorbing materials. With the deuteron streams, Lawrence has produced radioactive isotopes of many of the different elements known in the periodic table. In many cases the yields of the radioactive substances are quite large; as for example, a day's bombardment of sodium metal with 20 microamperes of 5 million volts deuterons produces more than 200 milligrams equivalent of radio-sodium, i.e. an amount of radio-sodium having a γ-ray activity equivalent to that of 200 milligrams of radium. That such large amounts of radio-active forms of many of the elements can be manufactured in the laboratory is of immense importance in opening up new avenues of research both in the physical and in the biological sciences. Many striking results have been obtained by Lawrence himself and his co-workers, while of course, similar work on nuclear transformations is being carried out in different physical laboratories of the world by other methods as well. But the cyclotron holds a unique position in that it can provide very large yields and possesses potentialities of even greater developments which stagger the imagination of the world.

Most of Lawrence's researches were encouraged by substantial public support. The Federal Telegraph Company donated the steel castings of the magnet. The Research Corporation and the Chemical Foundation provided funds for the construction and installation of the magnet and accessory apparatus, while the operating expenses were met by the University Research Board. But above all it was the genius and the single-minded devotion of Prof. Lawrence that overcame all the practical difficulties and brought to a very successful fruition an idea that must well nigh have looked fantastic when it was originally conceived; no wonder, the world applauds.

B. DASANNACHARYA

Benares Hindu University,
November 20, 1939.

SEDOV ARCTIC EXPEDITION*

THE Second Anniversary of the drift of the Soviet ice breaker 'Sedov' fell on the 23rd October. The drift of this breaker which bears the name of the celebrated arctic explorer, Georgi Sedov, began on October 27, 1937, in the Laptev Sea at 75° 19′ N lat. and 132° 25′ E long. The bearings on October 20 were 80° 36′ N lat. and 26° 12′ E long.

From the astronomical and meteorological data collected, it has now been established that the ice moves along isobars. This conclusion is of much practical significance, for from the data relating to the distribution of atmospheric pressures in the Arctic basin it would be possible to determine the shift of sections of ice in the central Arctic region. The hypothesis that ice moves from East to West under the influence of winds in a circular clockwise direction, with its centre near the 'pole of inaccessibility' situated between 83° and 85° N lat., first enunciated by the Russian Arctic Expedition, headed by Toll in 1900–03, has now been confirmed. The cause for such a remarkable phenomenon is the existence of more or less permanent stretches of open water or fissures in the region north of Greenland and north of the New Siberian Islands and Wrangal Island. The depth soundings taken by the Sedov showed that at 86° 26′ N lat. and 39° 85′ E long. the depth was greater than 5,180 meters.

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ARTICLES

INHIBITION OF SWEET POTATO β-AMYLASE BY A POLYCATION

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ABSTRACT

A novel polycation was synthesized. Inhibition of partially purified sweet potato β-amylase by the polycation was studied. Fifty per cent inhibition was obtained at 6.5 mM. Kinetic studies indicate that inhibition was noncompetitive and reversible, and K_i was 7.3 mM. The Hill coefficient was 1, suggesting that the polycation interacts with the enzyme non-cooperatively. Thermodynamic studies on enzyme–polycation complex indicate that the reaction is exothermic. The negative value of ΔS indicates that the enzyme molecule undergoes conformational change during interaction with the polycation. The polycation also inhibits β-amylase derived from maize, wheat, barley and pea.

INTRODUCTION

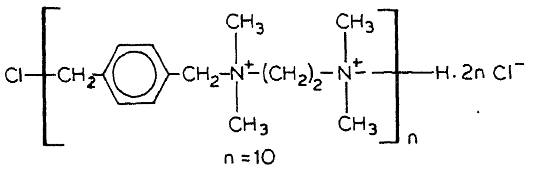
β-AMYLASE (α, 1–4 glucan maltohydrolase EC 3.2.1.2) catalyses the liberation of maltose from nonreducing ends of α, 1–4 glucan. The interaction of enzyme with substrates, products and inhibitors has been investigated by kinetic and spectroscopic methods[1]. Inhibition of soybean β-amylase by glucose[2], maltose[3] and cyclohexaamylase[4] have been demonstrated by kinetic studies.

Biologically important polyamines such as putrescine, spermidine and spermine are cationic substances, and have been shown to interact with DNA and RNA and to affect the activities of several enzymes[5–8]. It was reported that synthetic polycations also strongly interact with nucleic acids[9]. Recent studies with a polycation showed that it was a potential inhibitor of mammalian amylases[10, 11]. The present investigation was undertaken to study the inhibitory action of a synthetic polycation on partially purified sweet potato β-amylase.

MATERIALS AND METHODS

Sweet potato β-amylase was purified according to the procedure of Hegde *et al.*[12] The enzyme from wheat, maize, barley and peanut was extracted according to Sharma *et al.*[13]

α,α′-Dichloro-p-xylene (XDC) was purchased from AG Switzerland; *N,N,N′,N′*-tetramethylethylenediamine (TEMED) from BDH, England; and soluble starch and dimethyl formamide (DMF) from BDH, Bombay. The polycation shown below was synthesized according to the procedure of Rembaum and coworkers.[14, 15].



The polycation was homogeneous. All other chemicals used were of analytical grade.

Enzyme assay

Enzyme activity was measured according to the method of Bernfeld[16] using dialysed starch as substrate. The amylase activity is expressed in terms of micromoles of reducing sugar liberated by 1 ml of enzyme per minute at room temperature. Protein concentration was determined according to Lowry *et al.*[17] using bovine serum albumin as standard.

Effect of polycation on plant amylases

Table 1 shows that the polycation inhibits β-amylase from maize, wheat, barley and pea to different extents in a concentration-dependent manner.

Detailed inhibition studies were carried out with sweet potato β-amylase. Different concentrations of polycation with 0.5 ml of enzyme and 0.5 ml of 1%

Table 1 *Effect of polycation on activity of plant amylases*

Inhibitor conc. (mM)	Inhibition (%)			
	Maize	Wheat	Barley	Pea
2	16.22	31.26	35.24	41.03
4	48.76	51.57	55.61	48.72
6	70.28	70.32	70.12	54.52
8	70.28	76.57	88.24	—

starch in 0.01 M acetate buffer, pH 4.8, were incubated at room temperature for 3 min and the residual activity was measured as absorbance at 540 nm in a Hitachi spectrophotometer. From the inhibition data a linear Hill plot of log $(V_0 - V_i)/V_i$ vs log polycation concentration was plotted according to Johnson *et al.*[18] The type of enzyme inhibition by polycation was determined according to the method of Lineweaver and Burk[19] and the apparent inhibitor constant (K_i) was determined from a Dixon plot[20]. The Lineweaver–Burk plot was analysed by least square analysis[21]. Reversibility of polycation was studied by incubating the different concentrations of enzyme with two fixed concentrations of polycation and plotting the data in an Ackermann and Potter plot[22]. Thermodynamic parameters of enzyme–polycation complex were studied at different temperatures (0–30°C) and enthalpy (ΔH), free energy change (ΔG) and entropy (ΔS) were calculated according to Taketa and Pogell[23]. The energy of activation was calculated from an Arrhenius plot[24].

RESULTS AND DISCUSSION

Fifty per cent inhibition of partially purified sweet potato β-amylase by polycation was obtained at 6.3 mM (figure 1A). This showed that the polycation is a potent inhibitor of sweet potato β-amylase. Maximum inhibition was obtained at 10 mM. The Hill plot (figure 1B) is a straight line with slope 1, indicating noncooperativity, which is associated with most systems involving allosteric interactions[25]. The Lineweaver–Burk plot (figure 2) shows that the inhibition by the polycation is noncompetitive. K_i was found to be 7.3 mM. The Ackermann and Potter plot (figure 3) indicates that the polycation binds reversibly. The effect of temperature on the formation of enzyme–polycation complex was analysed in terms of thermodynamic parameters by assuming that K_i values at different temperatures represent association constants for enzyme–polycation complex formation. This assumption allowed calculation of the enthalpy change ΔH involved in the formation of the complex from a plot of pK_i as a function of the reciprocal of absolute temperature (figure 4A) as

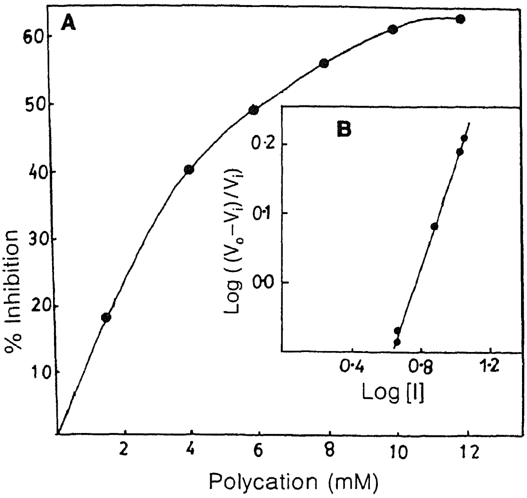


Figure 1. **A,** Inhibition of sweet potato β-amylase by polycation. **B,** Plot of $\log((V_0 - V_i)/V_i)$ vs log polycation concentration. V_0 is enzyme activity without polycation and V_i is activity in presence of polycation.

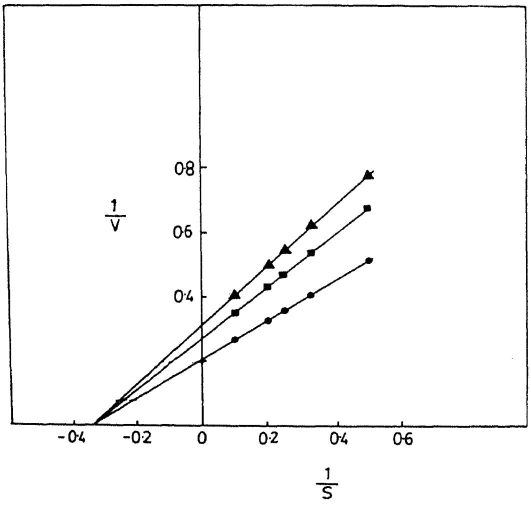


Figure 2. Lineweaver–Burk plot showing noncompetitive inhibition of sweet potato β-amylase by polycation. ●, No inhibitor; ■, 3 mM polycation; ▲, 5 mM polycation.

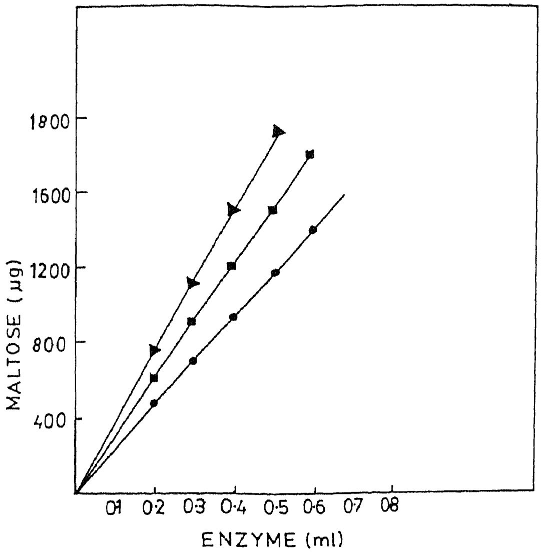


Figure 3. Ackermann and Potter plot. ▲, No inhibitor; ■, 2 mM polycation; ●, 5 mM polycation.

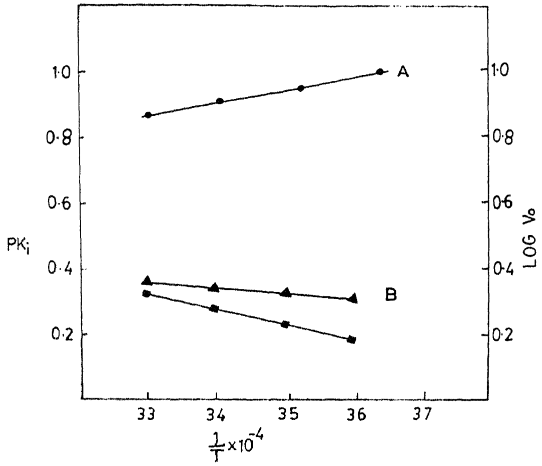


Figure 4. **A,** Van't Hoff plott. Values of K_i at different temperatures were obtained from Dixon plots. The molar enthalpy change ΔH (●) was calculated (plot of pK_i vs $\frac{1}{T}\times10^{-4}$) from the slope according to the equation $\Delta H = -4.57\times\text{slope}$. **B,** Arrhenius plot of log V_0 vs $\frac{1}{T}\times10^{-4}$. Energy of activation E_a was calculated for enzyme (▲) and enzyme with polycation (■) from their respective slopes according to the equation $E_a=\text{slope}\times2\times2.3$.

Table 2 *Thermodynamic parameters for interaction between the polycation and sweet potato β-amylase*

Temp (°C)	ΔS (cal/deg/mol)	ΔG (cal/mol)
0	−2.180	1305.8
10	−2.401	1221.4
20	−2.569	1148.1
30	−2.660	1093.2

described by Taketa and Pogell[23]. The calculated ΔH value of −1091 cal/mol indicates the exothermic nature of the reaction. The free energy change (ΔG) and entropy change (ΔS) for enzyme–polycation complex formation were calculated using the following equations:

$$\Delta G = -RT\ln k_i \quad \text{and} \quad \Delta S = \frac{\Delta H - \Delta G}{T}.$$

The results are summarized in table 2. The ΔS values were negative and remained constant throughout, indicating that the formation of inactive β-amylase–polycation complex involved marked conformational change in the enzyme. Energy of activation was calculated from figure 4B and is 506 cal/mol for enzyme alone and 920 cal/mol for enzyme plus polycation. The difference is required to break the noncovalent bonds of the enzyme–polycation complex.

Ikemiya[26,27] found that alkyldimethylbenzylammonium chloride was a potential inhibitor of β-amylase from malt, soybean and sweet potato. Pomeranz[28] reported that cetyl quaternary ammonium compounds were highly effective in inactivating amylases from pancreas, bacteria and fungi. Mulimani and Day[29] showed that the polycation inhibits *Bacillus subtilis* α-amylase; enzyme inhibition was achieved by electrostatic interaction between polycation and β-amylase. The quaternary nitrogen group of the polycation interacts electrostatically with essential anionic sites of the enzyme. The affinity of the polycation for the anionic sites of the enzyme has been shown to depend on the presence of the long-chain alkyl group.

Direct interaction between polyamines and protein kinases has been described, which might have led to conformational change in the binding site[30]. Inhibition of sweet potato β-amylase by polycation might also be due to a specific change induced by the binding of polycation. The positive quaternary nitrogen of polycation is specifically adapted to substrate binding region of β-amylase.

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AMINO ACID DECARBOXYLASES OF *ANCYLOSTOMA CEYLANICUM* AND *NIPPOSTRONGYLUS BRASILIENSIS*

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ABSTRACT

Dialysed homogenates as well as partially purified extracts of *Ancylostoma ceylanicum* and *Nippostrongylus brasiliensis* mediated decarboxylation of all twelve amino acids were examined. Maximum CO_2 was produced by glutamic acid, and minimum by leucine, phenylalanine and valine. γ-Aminobutyric acid and ethanolamine were identified as the decarboxylation products of glutamate and serine respectively. Evidence concerning the presence of true amino acid decarboxylases in the two parasites has also been collected. All the anthelminthics employed in the study were found inhibitory to glutamate decarboxylase, with slight variation in degree of effectivity.

INTRODUCTION

Ancylostoma ceylanicum and *Nippostrongylus brasiliensis* have recently been shown to produce CO_2 from a number of amino acids[1]. Similar information is available for a few other parasites[2,3]. These parasites however mediate active transamination also[1,3,4] and therefore are capable of converting amino acids into the corresponding ketoacids which may be additional source of CO_2. It is not yet clear whether the released CO_2 comes directly from amino acids or indirectly from their transamination products. In order to establish direct decarboxylation of amino acids, transamination must be made nonfunctional. This can be achieved by removing the required cofactors from the system. Accordingly decarboxylation has been studied with partially purified homogenates. Attempts have also been made to characterize the metabolic products of a few decarboxylases and to examine their susceptibility to certain known and candidate chemotherapeutic agents.

MATERIALS AND METHODS

Chemicals and reagents

U-^{14}C-labelled alanine, arginine, aspartic acid, lysine, glutamic acid, proline, serine and tyrosine; 1-^{14}C-labelled glycine, leucine, phenylalanine and valine; and 5-^{14}C-labelled glutamic acid were obtained from Bhabha Atomic Research Centre, Bombay. A kit of unlabelled amino acids was procured from Loba Chemie, Bombay. Mebendazole was from Cadila Laboratories, Ahmedabad; praziquantel from E. Merck, West Germany; niclosamide from Bayer AG, West Germany; and levamisole from Janssen Pharmaceutica, Belgium, as gift. Compounds 81/470, i.e. methyl-5(6)-[4-(2-pyridyl)]-piperazine carbamoylbenzimidazole-2-carbamate[5]; 82/437 i.e. 2,2′-dicarbomethoxylamino-5,5′-dibenzimidazolyl ketone[6]; and 83/148, i.e. methyl-5(6)-[2,5-dimethyl-3-methoxycarbamylfuranobenzimidazole]-2-carbamate[7] were products of the Medicinal Chemistry Division, CDRI, Lucknow.

Parasites

A. ceylanicum were isolated from golden hamsters with a 18–20-day-old infection and *N. brasiliensis* from rats infected 9 days earlier. The worms were collected in normal saline and thoroughly cleaned.

Preparation of homogenate

A 5% (w/v) homogenate of worms was prepared in 1.15% KCl and centrifuged at 9000 *g* for 30 min. The supernatant was dialysed against several changes of 20 mM phosphate buffer, pH 7.4 and recentrifuged.

Column chromatography

The dialysed supernatant (10–20 mg protein) was loaded on a DEAE-Sephacel column (1 × 40 cm) equilibrated with 20 mM phosphate buffer, pH 7.4. Unadsorbed proteins were washed off the column by running 100 ml of equilibrating buffer. The adsorbed proteins were eluted with the same buffer containing 0.5 M NaCl. Four ml fractions were collected at a flow rate of 1 ml/min. Fractions of two peaks containing significant amount of protein were pooled, dialysed against buffer, and assayed for decarboxylation.

Assay of decarboxylation

Suitable aliquots of homogenate of purified fractions were shaken at 37°C in 25 ml capped Erlenmeyer flasks containing (in 1.0 ml) 100 μmol phosphate buffer pH 7.4, 10 μmol $MgCl_2$; 1.0 μmol labelled amino acid (sp. activity 1 μCi/5 μmol). The liberated $^{14}CO_2$ was absorbed in 0.1 ml of 1 M methylbenzethonium (hyamine) hydroxide soaked up on filter paper wicks placed in the glass-wells which were suspended into the flasks through the caps[2]. After 2 h the reaction was terminated by injecting 0.1 ml of 1 M perchloric acid. The flasks were then shaken for an additional 30 min to allow the liberated $^{14}CO_2$ to be absorbed by the alkali. Appropriate controls without parasite fractions were run simultaneously. The filter papers containing absorbed $^{14}CO_2$ were counted for radioactivity.

Identification of reaction products

One hundred μl of enzyme preparation (concentrated 20-fold by immersible CX-10 ultrafiltration (Millipore, USA)) was incubated at 37°C with 1 μmol of labelled amino acid (5 μCi), and 200 units of penicillin-G and 200 μg of streptomycin. Total volume of the reaction mixture was 200 μl. After 0.5, 6 and 24 h, a 50 μl aliquot was removed and placed in a boiling water bath for 5 min. Twenty μl was applied on a Whatman paper No. 1 along with standards. The material was chromatographed for 18 h in *n*-butanol–acetic acid–water (4:1:1). After drying the paper in air, radioactive spots were visualized by autoradiography while the unlabelled standards were stained with ninhydrin. The areas corresponding to the standards were cut out from the chromatogram and counted for radioactivity.

Radioactivity counting

The samples were mixed with 10 ml of Bray's solution and counted for radioactivity in a Packard Tricarb scintillation spectrometer. Correction for quenching was applied using an internal standard.

Protein was estimated colorimetrically[8] using bovine serum albumin as standard.

RESULTS

Dialysed homogenates of both *N. brasiliensis* and *A. ceylanicum* catalysed decarboxylation of all the 12 amino acids were examined (table 1). *A. ceylanicum*, in general, produced greater amounts of CO_2 than *N. brasiliensis*. Among the amino acids, glutamate was decarboxylated to the greatest extent while the aromatic amino acids phenylalanine and tyrosine were less susceptible. Passage of the homogenate through DEAE-Sephacel resulted in distribution of proteins between unadsorbed and adsorbed fractions (figure 1). The adsorbed protein could be eluted with buffer containing 0.5 M NaCl. Surprisingly, both fractions decarboxylated all the twelve amino acids with little quantitative differences. This cannot be considered to be due to peak trailing since the separation of proteins into two distinct fractions is clearly evident from figure 1.

Formation of decarboxylation product, as judged by the product vs substrate ratio, increased with reaction time (table 2). For instance, the ethanolamine/serine ratio was very low 0.019 at 0.5 h but rose to 0.298 and 1.08 after 6 and 24 h respectively. Likewise, for [U-^{14}C]glutamate, the ratio of γ-aminobutyrate/glutamate increased from 0.032 at 0.5 h to 1.16 at 24 h. Similar observations were recorded for *A. ceylanicum* also.

All the anthelminthics tested reduced CO_2 liberation from glutamate. The maximum inhibition was by 25 μM Praziquantel of the *A. ceylanicum* enzyme (table 3). For *N. brasiliensis*, on the other hand, compounds 82/437 and 83/148 proved most effective.

DISCUSSION

Liberation of CO_2 from amino acids appears to be a common feature of helminth parasites[1–3,9,10]. However, the question whether this CO_2 arises from direct decarboxylation of amino acids or from keto acids produced during transamination remains to be answered. The present results provide strong evidences in favour of direct decarboxylation of amino acids by *A. ceylanicum* and *N. brasiliensis*. The first convincing clue is obtained from the observation concerning the production of CO_2 in the presence of dialysed and partially purified parasite extracts (table 1). During these steps certain important cofactors are removed from the extracts. For example, an amino group acceptor like 2-oxoglutarate, pyruvate or oxalacetate is required for

Table 1 *Rate of $^{14}CO_2$ production from various amino acids by homogenate and partially purified extract of A. ceylanicum and N. brasiliensis*

	$^{14}CO_2$ produced (nmol/h/mg protein)					
	N. brasiliensis			*A. ceylanicum*		
Amino acid	Homogenate	Unadsorbed fraction	Adsorbed fraction	Homogenate	Unadsorbed fraction	Adsorbed fraction
[U–^{14}C] Alanine	7.5	9.5	11.66	45.97	141.05	43.05
U–^{14}C] Arginine	20.3	12.38	21.28	27.67	48.03	56.18
[U–^{14}C] Aspartate	18.6	24.94	31.29	38.69	107.49	177.31
[U–^{14}C] Glutamate	158.5	218.25	247.1	593.0	781.8	1222.0
[5–^{14}C] Glutamate	5.3	16.34	80.49	81.59	258.01	309.02
[1–^{14}C] Glycine	4.5	11.1	19.14	17.18	29.25	60.76
[1–^{14}C] Leucine	1.84	7.22	11.44	1.34	1.84	12.66
[U–^{14}C] Lysine	15.0	20.93	32.07	12.52	31.01	105.76
[1–^{14}C] Phenylalanine	3.45	2.32	10.26	2.73	4.37	5.35
[U–^{14}C] Proline	18.6	22.70	36.70	46.13	99.03	142.99
[U–^{14}C] Serine	8.5	18.8	28.69	39.8	141.22	−44.85
[U–^{14}C] Tyrosine	0.95	2.73	10.64	5.22	12.16	11.51
[1–^{14}C] Valine	0.89	1.76	2.43	8.77	6.01	18.24

Data are mean of three experiments.

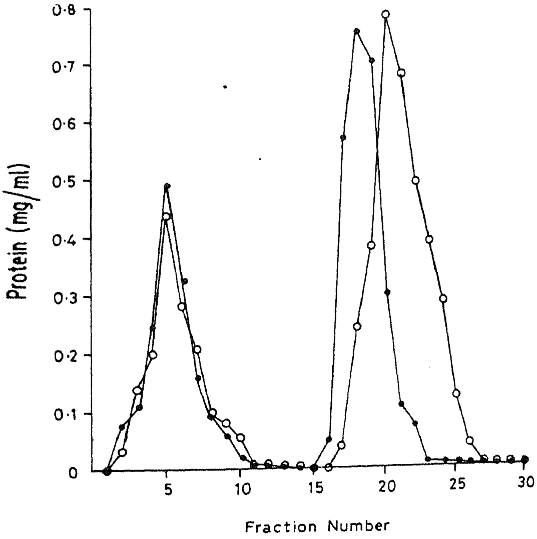

Figure 1. Elution pattern of soluble proteins of *A. ceylanicum* (○) and *N. brasiliensis* (●) on DEAE-sephacel.

transamination. Likewise, NAD, NADP or FAD is needed for dehydrogenases. In the absence of these factors, as in present case, transamination or dehydrogenation reactions cannot proceed. Minor amounts of tightly bound cofactors cannot support these reactions significantly. Hence, CO_2 evolved in presence of dialysed or partially purified extracts may originate directly from amino acids.

Table 2 *Accumulation of decarboxylated metabolites by activity of purified extract of N. brasiliensis and A. ceylanicum*

	Product/substrate ratio after		
Substrate	0.5 h	6 h	24 h
N. brasiliensis			
[5–^{14}C]Glutamate	0.012	0.080	0.31
[U–^{14}C]Glutamate	0.032	0.141	1.16
[U–^{14}C]Serine	0.019	0.298	1.08
A. ceylanicum			
[5–^{14}C]Glutamate	0.048	0.130	0.398
[U–^{14}C]Glutamate	0.032	0.251	1.261
[U–^{14}C]Serine	0.192	0.360	1.683

Data are mean of three experiments.

Table 3 *Effect of anthelminthics on partially purified glutamate decarboxylase of A. ceylanicum and N. brasiliensis*

	Inhibition (%)			
	N. brasiliensis		*A. ceylanicum*	
Anthelminthic agent	10 μM	25 μM	10 μM	25 μM
Mebendazole	11.04	23.65	14.59	27.23
Praziquantel	14.42	20.41	16.41	70.30
Niclosamide	15.25	36.74	28.49	46.17
Levamisole	15.76	34.83	16.25	38.20
81/470	12.81	10.37	12.34	25.70
82/437	34.23	41.26	16.21	49.11
83/148	38.61	44.79	17.18	24.68

Data are mean of 3 experiments.

Identification of ethanolamine, γ-aminobutyrate and 2-aminobutyrate as the reaction products provides further support for the existence of true decarboxylase(s) at least for two amino acids, serine and glutamate. Formation of both γ-aminobutyrate and 2-aminobutyrate suggests release of CO_2 from both carboxyl groups of glutamate. [5-^{14}C]Glutamate, when decarboxylated at the γ-carboxyl group, would yield unlabelled product. Hence labelled reaction product from [5-^{14}C]glutamate can be generated only in presence of α-decarboxylase. Further, generation of labelled CO_2 from [U-^{14}C]glutamate proceeds at nearly four times the rate from [5-^{14}C]glutamate. This can only be explained by liberation of more labelled CO_2 from the labelled γ-carboxyl group in [U-^{14}C]glutamate.

All the anthelminthics examined inhibit glutamate decarboxylase of both parasites. The very little difference in degree of effectivity between these compounds would seem to preclude a specific mode of action against this enzyme. Yet, the inhibition of *A. ceylanicum* enzyme by praziquantel is interesting, as the antischistosomal action of this drug has been attributed to interference with neuromuscular activity of the parasite[11]. Glutamate decarboxylase produces γ-aminobutyrate which is a known neurotransmitter.

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A STUDY OF ACID–BASE STATUS AND GASES IN BODY FLUIDS IN TWO SPECIES OF BOVINES

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ABSTRACT

A comparative study was undertaken on acid–base status and gases in arterial and venous blood and lumbar cerebrospinal fluid (CSF) in bovine species. The results revealed that arterial and venous blood pH, plasma bicarbonate, and base excess were significantly different between cow calves and buffalo calves. Bicarbonate content and pCO_2 of CSF did not vary between cow calves and buffalo calves, and were similar to those in man but lower than in dogs. It was concluded that acid–base components and gas parameters of CSF were more close to venous blood in pCO_2 and base excess but were similar to arterial blood in respect of pH and oxygen saturation. This signifies that information about acid–base parameters and gases in CSF can be obtained only by analysis of CSF samples and cannot be assessed from analysis of blood samples.

INTRODUCTION

BLOOD and cerebrospinal fluid (CSF) acid–base status and gases are important physiological parameters for diagnosis (in establishing pathophysiology of metabolic, gastrointestinal and cardiopulmonary disorders) and therapeutic purposes. The nature (respiratory or metabolic) and degree (moderate or severe) of acidosis and alkalosis determine their influence on cardiac rate, cardiac output, blood pressure and peripheral vascular resistance[1–3]. However, a perusal of the literature indicates that there is scanty information on these parameters in blood and CSF of animals. The present study reports the physiological status of acid–base parameters and gases in arterial and venous blood and CSF, and comparative evaluation of these parameters in cow calves and buffalo calves.

MATERIALS AND METHODS

Twelve healthy crossbred (Red-Dane × Sahiwal) and 12 buffalo calves of about one year of age were selected for the present study. The animals were kept on standard feeding and management practices[4]. Jugular vein and carotid artery exposed surgically were fitted with a siliconized catheter for collection of blood. The blood samples were drawn in heparinized air-tight disposable polypropylene radiometer syringes. Lumbar CSF was collected from comfortably controlled animals in calibrated glass capillary tubes and were immediately sealed with no air bubble or air column. The polypropylene syringes were provided with silicone sealing ring and cellulose fibre disk for impregnating sodium heparin (70 IU) compensated for Na^+, K^+ and Ca^{2+} binding. The samples were immediately analysed using a blood gas analyser (Radiometer ABL 30, Copenhagen, Denmark) for pH, partial pressure of CO_2 (pCO_2), pO_2, actual bicarbonate (HCO_3^-), total CO_2, actual base excess (ABE), standard base excess (SBE), standard bicarbonate (SBC), oxygen saturation (SAT), oxygen saturation (O_2 CT) and oxygen extraction ratio (OER). Arterial and venous carbonic acid (H_2CO_3) were calculated from pCO_2 and solubility coefficient of CO_2 in blood[5] using H_2CO_3 (mmol/l) = pCO_2 (mm Hg) × 0.03. OER was calculated using the formula[6] OER (%) = [(Arterial oxygen content (vol. %) − Venous oxygen content (vol. %)/Arterial oxygen content (vol. %)] × 100. The data were statistically analysed at 1 and 5% levels of significance by Student's *t* test.

RESULTS AND DISCUSSION

The mean arterial pH of cow calves (table 1) was lower than the reported value for Holstein–Friesian calves[7] but was very close to those reported for Ayrshire calves[8]. The difference in sampling techniques, method of analysis (rewarming of plasma or

Table 1 *Physiological values of acid–base variables and gases in arterial and venous blood and cerebrospinal fluid (mean ± SE) of cow calves and buffalo calves*

	pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3^- (mmol/l)	TCO_2 (mmol/l)	H_2CO_3 (mmol/l)	ABE (mmol/l)	SBE (mmol/l)	SBC (mmol/l)	SAT (%)	O_2CT (vol %)	OER (%)
Cow calves												
Arterial blood	7.392 ±0.005	38.14 ±0.64	87.94 ±2.68	22.91 ±0.51	24.04 ±0.53	1.144 ±0.02	−1.1 ±0.63	−0.87 ±0.65	23.00 ±0.49	96.36 ±0.34	15.01 ±0.07	29.24 ±0.43
Venous blood	7.351 ±0.008	46.63 ±1.36	38.60 ±0.85	24.23 ±0.71	25.60 ±0.75	1.339 ±0.04	−1.37 ±0.49	−1.47 ±0.51	22.88 ±0.56	69.75 ±1.64	10.75 ±0.25	—
CSF	7.326 ±0.012	44.58 ±1.61	70.56 ±2.38	22.62 ±0.89	23.99 ±0.92	1.337 ±0.04	−2.09 ±0.86	−2.32 ±0.94	22.20 ±0.91	91.58 ±1.64	— —	—
Buffalo calves												
Arterial blood	7.446* ±0.009	38.68 ±1.32	87.81 ±2.61	25.88* ±0.66	27.06* ±0.69	1.158 ±0.03	1.03* ±0.62	0.75 ±0.71	26.08* ±0.61	96.78 ±0.47	14.46 ±0.24	26.20 ±1.08
Venous blood	7.412* ±0.008	41.90* ±1.22	39.51 ±1.30	27.06* ±0.68	28.34* ±0.68	1.229** ±0.03	1.61* ±0.63	1.88 ±0.67	25.75* ±0.72	66.54 ±2.16	10.76 ±0.42	—
CSF	7.335 ±0.009	43.21 ±1.72	54.65* ±2.67	23.26 ±0.81	24.62 ±0.85	1.296 ±0.04	−1.95 ±0.69	−2.26 ±0.82	22.82 ±0.73	84.49* ±1.03	—	—

Difference between cow calves and buffalo calves; *significant at 1%; **significant at 5%.

serum separated at room temperature to 38°C; filling of dead space of syringe with oil[7], causing more solubilization of CO_2, degree of equilibration of which is not known[9]; and use of calorimetric method), and difference in age[10,11] might be responsible for the differences from the reported values. Mean plasma bicarbonate was lower than derived plasma bicarbonate in Ayrshire cattle[10]. Measurement of plasma bicarbonate may lead to overstatement of acidosis and alkalosis[12]. In the present study mean arterial standard bicarbonate value was similar to the ones reported by Donawick and Baue[11]. Standard bicarbonate, which too accounts for change in pCO_2 and small differences caused by unsaturation of blood, does not accurately reflect quantitative changes in metabolic components[1]. Standard bicarbonate and base excess or base deficit, which are quantitative estimates of surplus base or acid, are better than any one alone. In the present study base excess was slightly different from the reports of Donawick and Baue[11]. This difference in actual bicarbonate and base excess may affect the use of Siggard–Anderson nomograms, which are based upon normal pH, pCO_2 and bicarbonate of human blood. There is some error inherent in the construction and use of all nomograms by virtue of the assumptions that must be made[1]. It is assumed that blood-buffering capacity is the same for all individuals and under all conditions. The constants of the Henderson–Hasselbach equation (pK of H_2CO_3 and solubility coefficient of CO_2) have been shown to be variable[13,14]. The *in vivo* buffer slope is also altered because of variation in blood to extracellular fluid volume ratio[15] and variations in H^+ or HCO_3^- distribution between extracellular fluid and intracellular fluid spaces[16]. Due to differences between buffering capacity of blood *in vitro* and *in vivo*, errors may be made when *in vitro* nomograms are applied to whole patient measurements[17] with actual haemoglobin concentration[18]. Variation of as much as 7 mEq/l in base deficient values in *in vitro* curves and *in vivo* measurements[19] which are identical for man and dog are very similar[20], but its reliability in bovines is not known. Total CO_2 content in arterial blood in the present study was also lower than the reported values[8,10]. Arterial pH, pCO_2, pO_2 and bicarbonate contents of buffalo calves agree with the findings of Singh *et al.*[21] Arterial and venous pH, HCO_3^-, TCO_2 and ABE were significantly ($P < 0.01$) different between cow calves and buffalo calves.

The pH of CSF is mainly dependent on pCO_2 and bicarbonate content. The pH of CSF of cow calves and buffalo calves were close to the values reported by Singh *et al.*[21] pCO_2 and bicarbonate content of CSF did not vary between cow calves and buffalo calves and were similar to the levels in man[22] but were lower than those in dog[22-24]. The oxygen saturation of CSF of cow calves was higher ($P < 0.01$) than that of buffalo calves. The higher saturation in cow calves may be due to higher CSF pO_2 and higher OER in cow calves. The large differences in pO_2 gradients between CSF compartments in the two bovine species are higher than in human lumbar CSF[25]. Venous blood may provide a better evaluation of the status of interstitial fluid of tissues than arterial blood[26,27]. CSF and brain interstitial fluids are considered to be of the same ionic strength[28]. But CSF was closer to venous blood in pCO_2, ABE and SBE, and similar to arterial blood in respect of pH, pO_2, TCO_2 and SAT. Therefore consistent and meaningful information about the acid–base status of CSF can be obtained by analysis of CSF and cannot be assessed from arterial or venous blood.

CONCLUSION

Venous blood analysis for acid–base variables and gas tension may be used only for limited therapeutic purposes. For proper diagnostic purposes, and for establishing pathophysiology of metabolic, gastrointestinal and pulmonary disorders, analysis of both arterial and venous blood is essential.

17 October 1988; Revised 13 February 1989

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SHORT COMMUNICATIONS

PETROLOGY OF THE BRICK-RED ROCK AND ALBITITE FROM THE SOUTHERN CLOSEPET GRANITE REGION

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THE Archaean Closepet granite (~2500 Ma) is a polyphase body intruding into the peninsular gneiss complex and the associated supracrustal rocks. The granite outcrop runs at least 350 km (lat 12° 15′ N to 14° 15′ N) with an approximate width of 20–25 km and cuts across the regional metamorphic structure. Earlier workers[1–4] who have studied the Closepet granite suggested a metasomatic origin. However, field observations in the southern end of the granite suggest that anatexis of Peninsular gneisses has led to the formation of granite melt[5,6]. Within the southern Closepet granite there are components with distinct mode of occurrence, colour and mineral composition. One of these lithologies is named brick-red rocks[2]. However, there is no information about their petrological and geochemical characteristics and genesis. The present paper records for the first time the occurrence of plagioclase–epidote–calcite-rich rock named albitite. The aim of this paper is to discuss the petrogenetic aspects of brick-red rocks as well as the albitite and their relationship with the associated Closepet granite.

Brick-red rock and albitites are generally found within the granite and occasionally at the margins of the granite outcrop. They predominantly occur at dolerite dyke margins.

Brick-red rock commonly occurs as sheet-like bodies forming ridges and are strongly weathered. They are deep red and have a porphyritic texture and contain large K-feldspar megacrysts which constitute up to 80% of the rock. The K-feldspar megacrysts are red and their abundance generally increases from the margin to the centre. Grass-green chlorite is abundant in the matrix. A number of epidote veins cut across the brick-red rocks. A gradational transition from the porphyritic granite (most voluminous phase of the Closepet granite) to the brick-red rocks can be observed. Occasionally hydrothermal quartz veins are found close to the brick-red rock outcrops in which the quartz crystals contain inclusions of opaque minerals.

The albitite is found in the vicinity of Ramanagaram close to the brick-red rock outcrops. The albitite is light-green and exhibits porphyritic texture with large alkali feldspar megacrysts. The megacrysts are white to cream-coloured and constitute up to 70% of the rock. Green epidote is abundant in the matrix. A gradational transition from the porphyritic granite can be recognized from the margin to the centre of the albitite outcrop.

Brick-red rocks

Brick-red rocks are megacrystic, and both the megacrysts and the ground mass are red. The presence of iron oxide provides red colouration to the rock. Modal composition of the brick-red rock is given in table 1.

In thin section the brick-red rock exhibits an inequigranular porphyritic texture and has the mineral assemblage quartz–plagioclase–K-feldspar–chlorite–calcite–grunerite.

Quartz is a minor phase occurring as anhedral grains. Plagioclase (An_{10-12}) occurs as subhedral grains with moderately developed albite twinning. In some instances plagioclase is moderately sericitized with abundant associated calcite. K-feldspar occurs as large anhedral grains and occasionally contains oriented euhedral inclusions of plagioclase. Grass-green chlorite occurs as anhedral grains. Calcite occurs as anhedral plates and has a rim of iron oxide. Brownish grunerite is found as radiating needles in association with calcite and iron oxide. Accessories such as zircon and apatite occur as inclusions in feldspars.

Albitite

Modal composition of the albitite is also given in

Table 1 *Modal composition of brick-red rock and albitite from the southern Closepet region*

Brick-red rock		Albitite	
K-feldspar	76.40	Albite	72.88
Plagioclase	6.10	Epidote	17.83
Chlorite	6.20	Clinozoisite	3.53
Calcite	4.5	Calcite	2.40
Quartz	2.2	Accessories	3.33
Accessories	1.4		
	100.00		99.97

table 1. In thin section the albitite exhibits an inequigranular porphyritic texture. The mineral assemblage consists of plagioclase–epidote–clinozoisite–calcite.

Plagioclase (An_{10-12}) is the most abundant phase and occurs as subhedral grains with moderately developed albite twinning and exhibits undulose extinction. Plagioclase is weakly sericitized and contains inclusions of epidote. K-feldspar is a minor phase and is found as anhedral plates. Epidote occurs as subhedral to anhedral plates and frequently shows lamellar twinning. Clinozoisite is anhedral and found as radiating needles. Anhedral plates of calcite are associated with plagioclase. The accessory phases such as zircon and apatite occur as inclusions in plagioclase and epidote.

Major and trace element analyses of the brick-red rock and the albitite were performed by XRF[7]. Samples of the Closepet granite close to the brick-red rock and albitite were also analysed for comparison. The analytical data are given in table 2.

The brick-red rock contains low SiO_2 (58.99%) and Na_2O (2.41%), high Al_2O_3 (16.21%) and very high K_2O (9.54%) compared to the Closepet granite. In trace elements the brick-red rock is depleted in Sr (248 ppm) and enriched in Rb (208 ppm). The brick-red rock shows similarity with the Closepet granite in the abundance of high field strength elements (HFS) such as Ti, Zr and Y and LREE.

Table 2 *Major and trace element composition of the brick-red rock and albitite, and Closepet granite close to these samples*

	Albitite	Brick-red rock	Closepet granite	
Major elements (%):				
SiO_2	60.93	58.99	66.47	71.17
TiO_2	0.71	0.62	0.65	0.59
Al_2O_3	18.85	16.21	15.54	14.01
Fe_2O_3	0.64	0.26	0.86	0.45
FeO	2.56	3.02	3.46	1.79
MnO	0.02	0.05	0.08	0.05
MgO	—	0.79	1.30	0.04
CaO	5.16	3.00	3.20	2.20
Na_2O	11.06	2.41	4.36	3.64
K_2O	0.19	9.54	3.26	4.24
P_2O_5	0.41	0.28	0.22	0.13
Total	100.53	95.17	99.40	98.31
Trace elements (ppm):				
Ba	1936	764	797	949
Rb	—	208	79	55
Sr	501	248	598	576
Zr	325	310	305	332
Y	33	29	30	26
La	106	120	104	123
Ce	169	175	159	181

The albitite has low SiO_2 (60.93%) and K_2O (0.19%). MgO is totally absent and Al_2O_3 (18.85%), CaO (5.16%) and Na_2O (11.06%) are very high compared to the Closepet granite. Among trace elements Rb is totally absent and enrichment of Ba (1936 ppm) is observed. The albitite also has high concentration of HFS elements and LREE, which is similar to the Closepet granite.

The mesonormative compositions of the brick-red rock, albitite and the associated Closepet granite were plotted on a Qz–Ab–An–Or tetrahedron (the granite system) for the condition $P_{H_2O} = 5$ kbar[8]. The brick-red rock and albitite fall, respectively, in the K-feldspar and plagioclase solidus volume, while the Closepet granite samples fall in the plagioclase + quartz + K-feldspar liquidus volume. The mesonormative compositions were also plotted on the two projections from the fourth face apex of the granite system. The Closepet granite samples fall close to the cotectic curve while the brick-red rock and the albitite fall outside the field.

The gradational transition from the Closepet granite to the brick-red rocks and albitite suggests a metasomatic origin for the latter. The hydrothermal quartz veins found very close to the brick-red rock and albitite may represent the SiO_2 removed during metasomatism. From petrographic observations it remains difficult to fix the origin of brick-red rock and albitite. However, the presence of low-temperature hydrothermal minerals favours a metasomatic origin. The major element data, particularly the very high K_2O (9.54%) in brick-red rock and Na_2O (11.06%) in albitite, strongly support a metasomatic origin. Composition plots for the granite system[8] envisage a magmatic origin for the Closepet granite and metasomatic origin for the brick-red rock and albitite. The HFS elements and LREE, which are considered to be immobile during secondary processes, are highly significant. The similarity in the abundance of HFS elements and LREE in the Closepet granite, brick-red rock and albitite indicate derivation of the brick-red rock and albitite from the Closepet granite by extensive metasomatism.

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NEOMERIS VAN-BOSSEAE HOWE—A NEW RECORD ALONG THE INDIAN COAST

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TWO species of *Neomeris* have so far been reported from the Indian coast, viz. *N. annulata* Dickie and *N. dumetosa* Lamoun. *N. annulata* has been reported from various localities of India by various authors, while *N. dumetosa* has been listed by Chacko[1] from the Krusadai Island. During a survey in the Lakshadweep islands *N. van-Bosseae* was found growing in the lower mid-littoral zone in December. Details of habit and habitat, morphology and reproduction are given below.

Habit and habitat

Thalli were found growing on calcareous rocks and on dead corals. Thalli are light-green and strongly calcified in the lower region and weakly in apical region.

Morphology

Plants are in clusters and attain a height of 1.5–2.5 cm (figure 1). The main axis gives rise to 28–40 whorls of laterals along the entire length. These primary branches are 460–580 µm long. Secondary branches arise in pairs 340–400 µm long (figure 2a). The inflated apex of secondary branches is broad and conical, 180–220 µm (figure 2b). Secondary

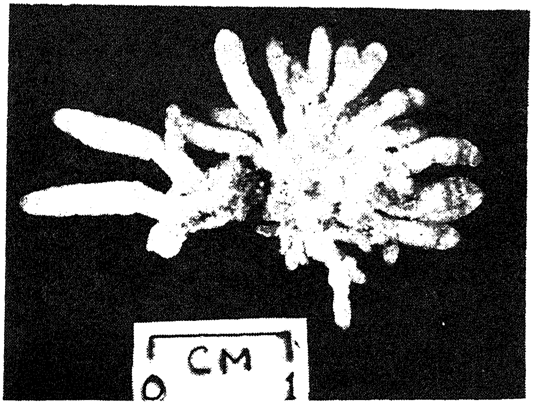

Figure 1. *Neomeris van-Bosseae*—Habit.

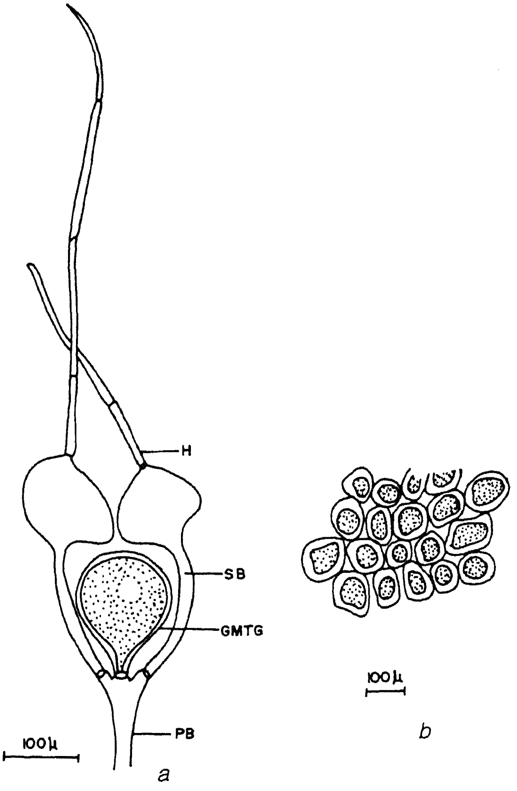

Figure 2. *N. van-Bosseae*: **a,** branching of primary (PB) and secondary (SB) order enclosing globular gametangium (GMTG), secondary branches bearing hairs (H); **b,** surface view of secondary branches.

branches terminate in a segmented, deciduous hair only at the apex, measuring about 1.0–1.5 mm in length (figure 2a).

Reproduction

Cametangia are pedicellate and produced on the primary branches, globose in shape. These are free even though strongly calcified and 88–110 μm in diameter. Each one bears a single spherical cyst.

This alga differs from *N. annulata* in having globose and free gametangia; in *N. annulata*, the gametangia are ovoid and cemented together in annular rows by a calcareous sheath.

The above account is in agreement with Egerod[2].

16 January 1989

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CYANOSTYLON CYLINDROCELLULARE GEITL. & RUTTN., A BLUE-GREEN ALGA NEW TO INDIA

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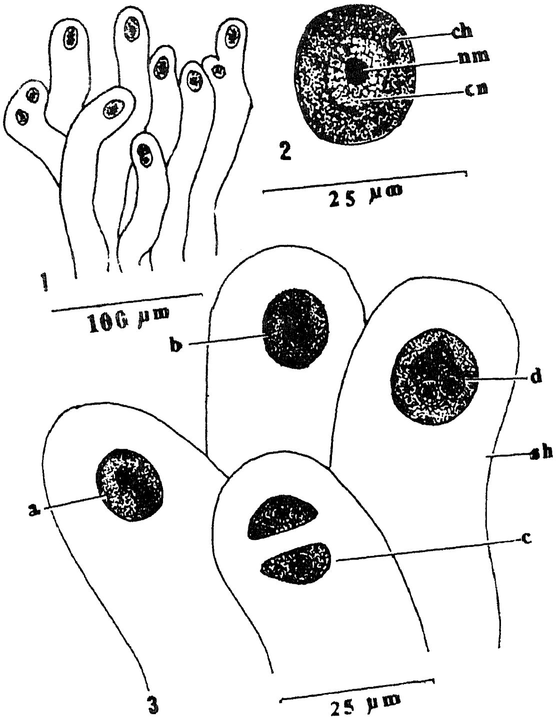
THE terrestrial blue green alga *Cyanostylon* has been collected from the cuttings of red rocks near Quilon, Kerala State. It was found growing along with *Zygogonium*, *Scytonema* and *Stigonema*. This monotypic genus has been described from Europe[1, 2]. So far there is no record from Asia.

Cells are uninucleate and colonial; colony gelatinous, macroscopic, irregular with spreading patches, 2–8 mm in diameter, slightly yellowish-brown. Each cell is capable of secreting mucilage only on one side and due to this unilateral secretion the cell is pushed towards the opposite direction resulting in the formation of a cylindrical stalk-like structure (figure 1). The finger-shaped mucilage strands may be branched or unbranched and contain one or more cells. The mucilage sheath is homogeneous and 23.3–26.6 μm in diameter. The bases of several such strands form a mass of mucilage.



Figures 1–3. *Cyanostylon cylindrocellulare* Geitl. & Ruttn. **1**, A few cells with cylindrical stalk. **2**, A single cell. **3**, Cells with division stages. (ch, Chromoplasm; cn, central nodule; nm, nuclear material; sh, sheath; a–d, stages in division.)

Cells are oval or spherical with a diameter of 12.4–18.6 μm. The protoplast is clearly differentiated into an outer granular chromoplasm and an inner transparent centroplasm with distinct nuclear material (figure 2). Reproduction is by binary fission and different stages of division are found in the same colony (figure 3). The nuclear material elongates and divides into two, followed by division of chromoplasm, resulting in the formation of two hemispherical daughter cells (figure 3c). In certain cells the division of the chromoplasm is delayed and 2–5 daughter centroplasmic structures are found in the same cell.

The cell is prokaryotic; still, it is more advanced due to the presence of a spherical transparent centroplasm with prominent nuclear material. Due to its peculiar habitat and structure Fritsch[3] suggested that it should be placed in a separate family. More cytological studies are required to confirm its position.

21 January 1989

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NIESSLIA EXILIS AND *PHYLLACHORA BALANSAE*: TWO NEW RECORDS FOR INDIA

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DURING a survey of microfungi the authors collected two ascomycetes hitherto unrecorded from India[1,2]. A brief description is given below:

Niesslia exilis (Albertini & Schweinitz ex Fries) Winter, Robenhorsts Kryptogamen Flora Deutschland 1 (2): 196 (1885).

Perithecia globose, superficial, gregarious, minute, brown to dark-brown, 100–120 μm across, covered with radiating, stiff, nonseptate, brown to dark-brown setae, numerous, 60–90 × 2.5–4.0 μm. Asci cylindric-clavate, numerous, arise in cluster, unitunicate, hyaline, briefly stipitate, 8-spored, 24–36 × 4.0–6.5 μm. Ascospores irregularly biseriate, hyaline to very light yellow in cluster, cylindric to fusoid, 1-septate, smooth, round ends, 5.0–6.5 × 1.5–2.0 μm (figure 1).

On dead leaves of *Lagerstroemia flos-reginae*, J. N. Agric. Univ. Campus, Jabalpur, India, 30.8.1986, Leg. N. D. Sharma.

The type specimen has been deposited in the Herbarium, CAB International Mycological Institute, Kew, No. 311974.

Niesslia pusilla (Fries) Schroeter, *N. mulleri* Ramachandra Rao and *N. anacardiae* Sathe & Mogarkar was the only three species of *Niesslia* Auerswald apud Gonnermann and Robenhorst recorded from India so far. We collected two species on the same host leaves. The perithecia in *N. mulleri* Ramchandra Rao are pear-shaped with robust, curved and 7–9 setose (figure 2). In the present collection perithecia, asci and ascospores are slightly different[3].

Phyllachora balansae Speg. in *Fung. Guar. Pug.* I: 110 (1883); *Syll. Fung.* IX., 1009, 1891.

Spots black, epiphyllous, separate, distributed all over the leaf, circular, up to 2 mm; stroma black, round, erumpent, amphigenous, generally epiphyllous, scattered 0.5 to 2 mm; 1–4 perithecia in a stroma, perithecia ostiolate, immersed, bowl-shaped, 200 to 600 μm; asci saccate, briefly stipitate, paraphysate, 1–2-seriate, 8-spored, 40–65 × 8–16 μm; ascospores hyaline, subglobose, thick-walled, 5–11 × 5–9 μm, av. 10 × 8 μm (figure 3).

On living leaves of *Cedrela toona* Roxb. (Meliaceae), Pachmarhi, India, October, 1968, Leg. N. D. Sharma.

The specimen has been deposited in the Herbarium, CAB International Mycological Institute, Kew, No. 138925.

The authors are grateful to Dr D. L. Hawksworth, Director, and Dr B. C. Sutton, Head, Taxonomic and Identification Service, CAB International Mycological Institute, Kew, England, for their help in the identification of the fungi.

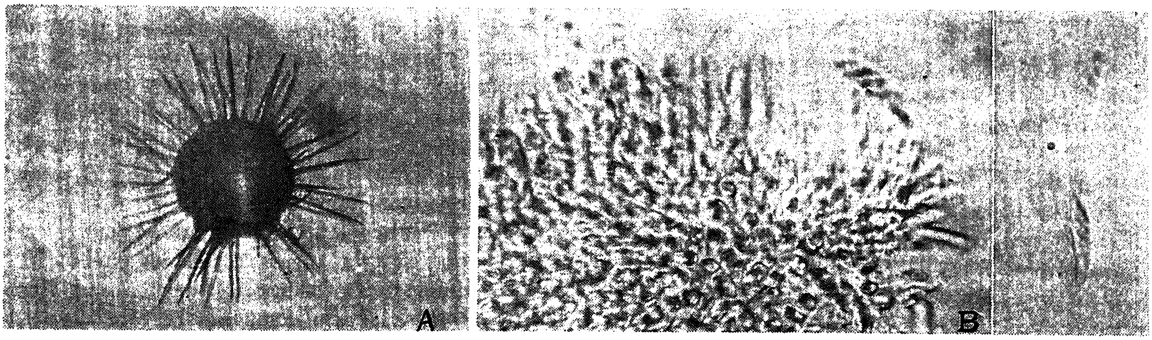

Figure 1. *Niesslia exilis.* **A,** Perithecium; **B,** asci.

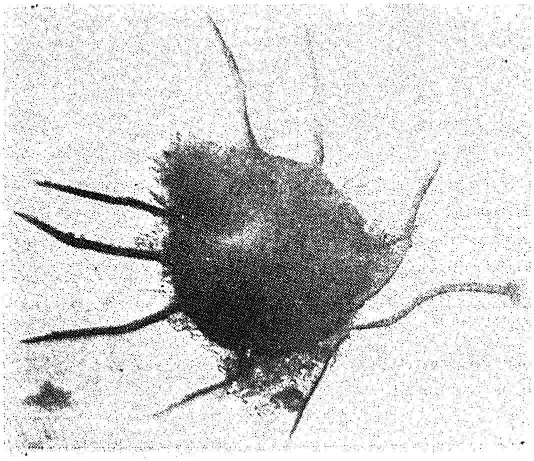

Figure 2. *Niesslia mulleri*—perithecium.

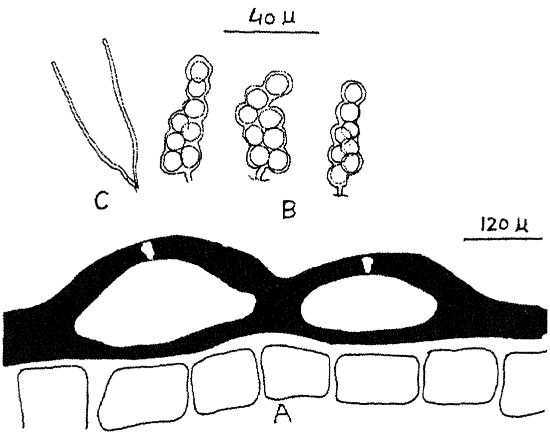

Figure 3. *Phyllachora balansae.* **A,** V.S. of ascostroma; **B,** asci with ascospores; **C,** paraphyses.

18 November 1989

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MORPHOLOGICAL AND ECOLOGICAL OBSERVATIONS ON *STIGEOCLONIUM FARCTUM*—CHAETOPHORALES, CHLOROPHYCEAE

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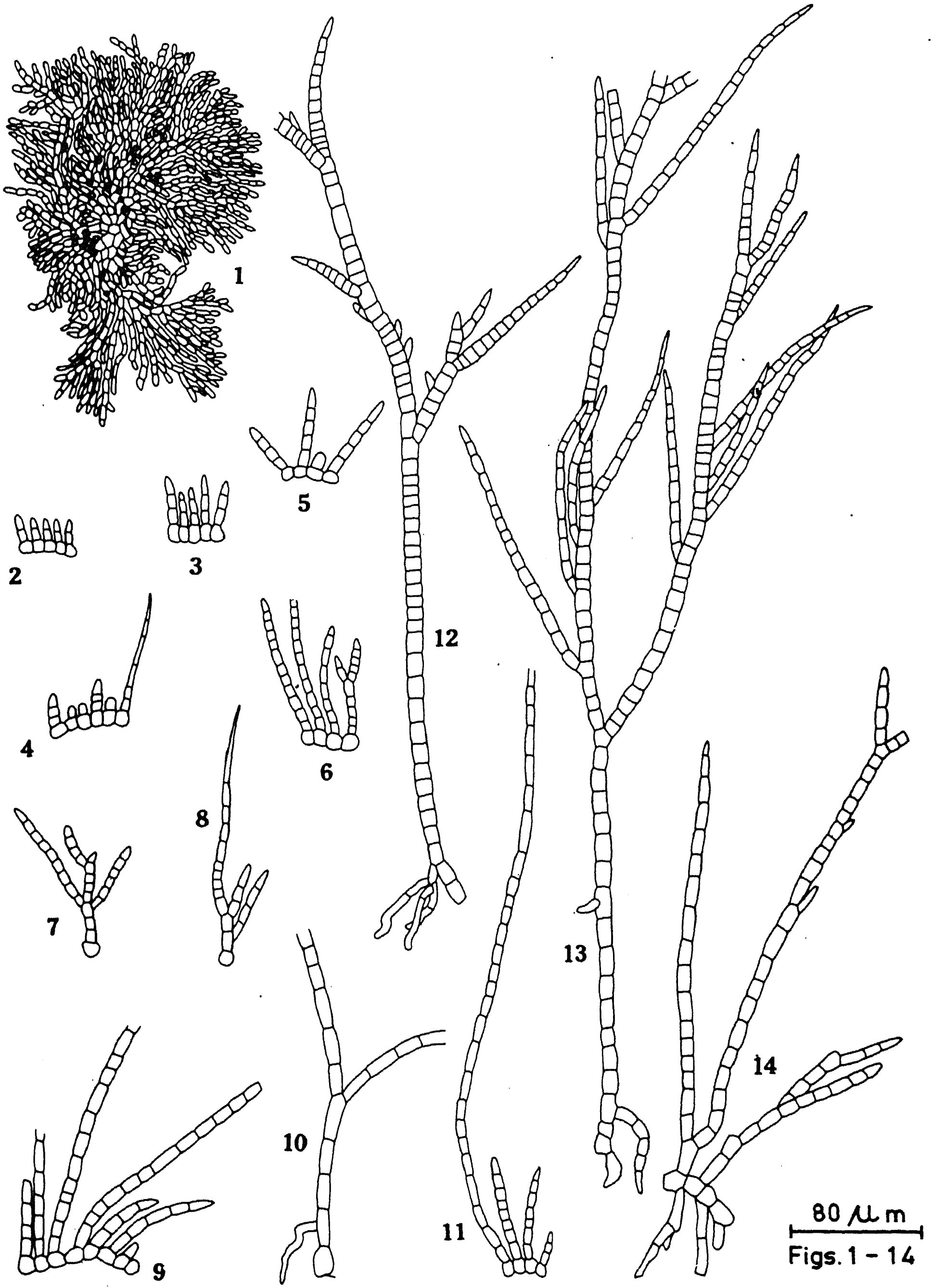
DURING hydrobiological studies of freshwater ponds of Lucknow, the author observed *Stigeoclonium farctum* with enigmatic morphology. It could not therefore be identified for a long time and was thought to be the vegetative thallus of *Coleochaete pseudosoluta* (an oogamous chaetophoralean member). Its morphological plasticity and physicochemical preferences are described here.

S. farctum was found growing epiphytically on submerged macrophytes (*Hydrilla*, *Chara* and *Ceratophyllum*) and submerged discarded polythene sheets. Thallus was heterotrichous, consisting of well-developed prostrate and erect systems. The prostrate system was composed of creeping filaments of restricted growth, giving rise to prostrate lateral filaments in an irregular manner, which rebranched, becoming as long as the main filament. The lateral prostrate filaments were so close to one another that they appeared as a pseudoparenchymatous disk, like the vegetative thallus of another discoid chaetophoralean member, *Coleochaete pseudosoluta* (figure 1). The prostrate cells in the central region were more or less angular or isodiametric but somewhat cylindrical towards the periphery. After a few months, some cells of the mature prostrate system started to give rise to erect filament initials, which looked like small beads on the surface of the prostrate thallus (figure 1). Later, these cells developed into short erect filaments (figures 2–8). Again, after a few months, the thallus looked different altogether like *Stigeoclonium*, with an extensively developed erect system with a range of morphological variability, ranging from unbranched to alternately branched, unilaterally branched to dichasially branched filaments (figures 2–14). Branch tips ended bluntly (figures 2, 3, 5, 6 and 7) or in multicellular hairs (figures 4, 8).

Both prostrate system cells (figure 14) and erect system cells (basal) gave rise to rhizoids (figures 10, 12 and 13). Rhizoids developing from terminal cells of the prostrate system could not be observed (*cf.* ref. 1).

The prostrate system cells in the central region



Figures 1–14. *Stigeoclonium farctum.* **1,** Well-developed prostrate system with young erect filament initials; **2–14,** different stages in the development of erect filaments.

were more or less angular or isodiametric, but towards the periphery they were somewhat cylindrical. In general, prostrate system cells were 6–9 (12.5) μm broad, and 5–10 (14) μm long. The erect

Table 1 *Physicochemical tolerance limits of S. farctum*

Parameter	Concentration (mg/l) (except 1 and 2)
pH	7.5–8.5
Temperature	19–34°C
Dissolved oxygen	3.2–11.2
Total organic matter	7.7–115.2
Total alkalinity	24.0–208.0
Alkalinity to phenolphthalein	ND–24.0
Total hardness	36.0–176.0
Calcium	16.0–76.0
Magnesium	1.0–24.3
Carbonate	ND–12.0
Bicarbonate	24.4–170.8
Chloride	28.0–108.0
Free and saline ammonia	ND–13.0
Albuminoid ammonia	ND–10.8
Nitrate nitrogen	1.8–10.7

ND, Not detectable.

system cells were cylindrical, 4–6 (8) μm broad and 5–15 μm long in young filaments, and 5–27.5 μm long in mature filaments.

The alga has been identified as *Stigeoclonium farctum*. It resembles 'strain 5-30' (in width of erect filament cells) and 'strain 7-17' (in nature of hair, presence of extensively developed erect filaments and the length of erect filament cells) of Cox and Bold[1].

Monthly water analysis and studies on Chaetophorales of four freshwater ponds of Lucknow during a whole year revealed that *S. farctum* could not grow throughout the year in all the ponds. In pond I, it was present from August to June, in ponds II and III from October to June, and in pond IV only during September to November. The first three ponds are situated in villages and receive different types of effluents while pond IV is situated in the city and receives urban and industrial effluent. The ranges of physicochemical factors favourable for *S. farctum* are given in table 1 (ref. 2).

The author thanks UGC, New Delhi, for financial assistance.

16 February 1989

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HELICOUBISIA CORONATA, NEW TO INDI

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DURING a study of fungi associated with decompo ing leaf litter of *Eucalyptus* species in South India helicosporous fungus was collected from dead falle leaves of *E. tereticornis* and subsequently identifie as *Helicoubisia coronata* Lunghini & Rambell This rare and interesting helicosporous hyphomycet was originally collected on dead stems from th Ivory Coast by Lunghini and Rambelli[1]. Since the further collections of this monotypic fungus have no been reported. Our collection represents the firs made outside the type locality and constitutes valuable addition to Indian fungi. A brief descriptio of the fungus is given below.

Colonies effuse, hairy. Conidiophores macronematous, scattered, straight, simple, dark brown below, paler above, 6–9-septate, up to 110 μm long 5–7 μm thick at the base and 3–4 μm thick towards the apex. Conidiophores terminating into 3–4 sporogenous cells bearing short denticles, 4–6 × 3–5 μm, olivaceous brown. Conidia acropleurogenous, coiled,

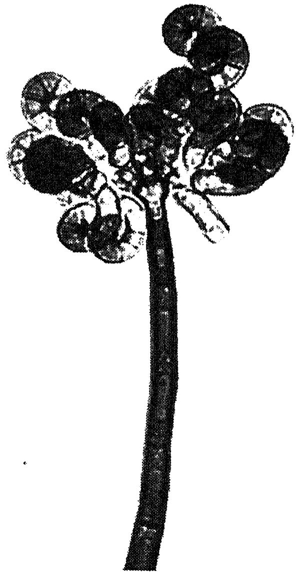

Figure 1. *Helicoubisia coronata* Conidiophore with young and mature conidia (× 700).

smooth, pale brown, 10–13 µm in diameter, rounded at the apices, narrowly truncate at the base, 6–8-septate, 4–5 µm thick.

Collected from leaf litter of *E. tereticornis*, Vandalur (Tamil Nadu), India, M. Dorai, May 1986, Herb. MUBL No. 3094.

The authors are thankful to Prof. R. D. Goos, University of Rhode Island, USA, for help in identifying the fungus and to UGC, New Delhi, for financial assistance.

9 March 1989

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PACHYKYTOSPORA KOTL. & POUZ. (POLYPORACEAE): A NEW GENERIC RECORD FROM THE HIMALAYAS, INDIA

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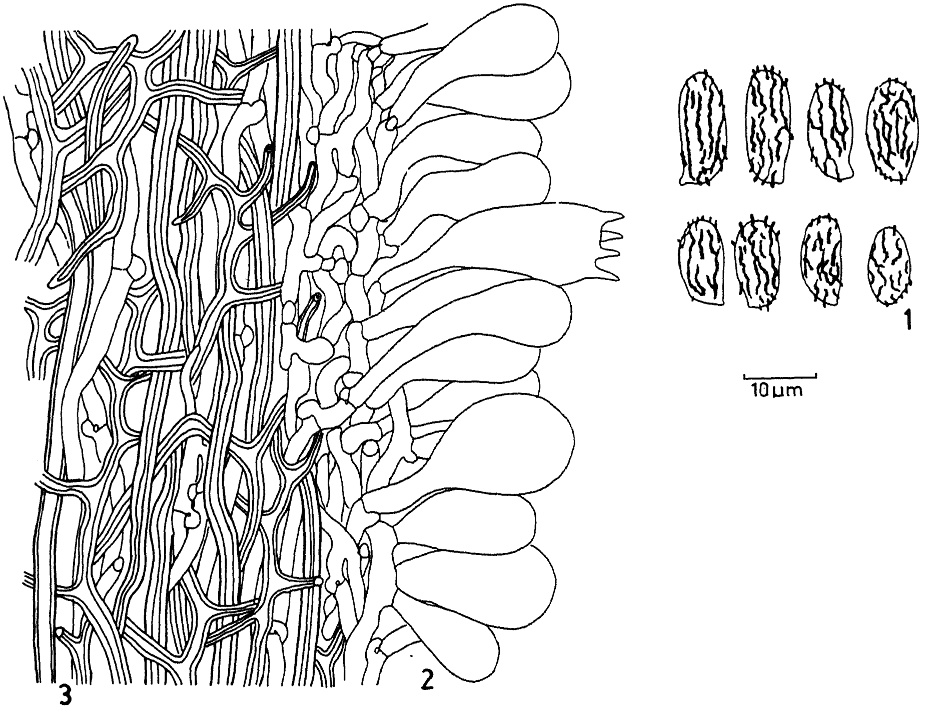
DURING trips to the eastern Himalayas from 1981 to 1985 many polypores were collected from various localities of Arunachal Pradesh. The present paper gives an illustrated account of *Pachykytospora papyracea* (Schw.) Ryv., a new record for India. The genus *Pachykytospora* is marked by resupinate fruit bodies, and di-trimitic hyphal system with ellipsoid basidiospores ornamented with warts or longitudinal striations staining strongly with cotton blue. *Perenniporia* Murrill is very close to *Pachykytospora* in having light-coloured fruit bodies, di-trimitic hyphal system with hyaline hyphae, and large spores. However, it has smooth, truncate basidiospores and dextrinoid hyphae.

Pachykytospora papyracea (Schw.) Ryv. *Norw. J. Bot.*, 19 (3–4): 233, 1972, (figures 1–3).

Fructification annual, resupinate, effused, adnate, pulvinate, soft coriaceous when fresh, brittle on drying, up to 4 cm long, 2.5 cm wide and 3 mm thick. Margin white to cream, concolorous with the pore surface, thinning, sterile, up to 2 mm wide. Pore surface white to cream when fresh, pale brown on drying; pores round to angular, 3–4 per mm, 238–450 µm in diameter; pore mouth velutinate; tubes in one layer white, up to 2 mm deep in section; dissepiment entire, thick. Context white to cream,



Figures 1–3. *Pachykytospora papyracea.* **1**, Basidiospores; **2**, hymenium; **3**, part of trama showing trimitic hyphal system in section.

soft, thin, homogeneous, non-xanthochroic, up to 0.8 mm thick.

Hyphal system trimitic; generative hyphae hyaline, thin to slightly thick-walled, septate, clamped, branched, cyanophilous, 1.2–2.5 μm in diameter; skeletal hyphae hyaline to subhyaline, thick-walled to almost solid with narrow lumen, aseptate, cyanophilous, 2–3.5 μm in diameter; binding hyphae hyaline, branched with short branches, aseptate, 1.6–3.0 μm in diameter. Cystidia and setae absent. Basidia hyaline, thin-walled, clavate, 2–4 spored, up to 12 μm wide. Basidiospores hyaline, thick-walled, ellipsoid ornamented with longitudinal striations, striae prominent and strongly cyanophilous, 9.5–14.5 × 4.5–6.0 μm.

Collection examined: India, Arunachal Pradesh, West Kameng, Rupa, 14 km from Rupa towards Shergaon; on *Rhododendron* stem; SSV 21756 (PAN); September 10, 1981.

Remarks: This is the first report of the occurrence of *P. papyracea* in the Himalayas, India. It appears to be of rare distribution in the Himalayas since my three years' explorations to various localities in the eastern Himalayas yielded only one collection. The Arunachal Pradesh collection is typical of the species and resembles the circumscription of the species as given by Ryvarden and Johansen[2].

P. papyracea is close to *P. tuberculosa* (Fr.) Kotl. & Pouz. However, the latter differs in having perennial, much thicker fructifications, pinkish pore surface, and larger 1–2 pores per mm.

The author thanks Dr Leif Ryvarden, University of Oslo, Norway, for identification and DST, New Delhi, for financial assistance.

24 January 1989

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GOMPHUS FLOCCOSUS—A NEW RECORD FOR INDIA

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DURING a survey of edible mushroom flora of North-Eastern India an interesting mushroom was collected from the pine and mixed forests of the East Khasi Hills and Chandel districts of Meghalaya and Manipur respectively in July, 1982 and 1984. Later it was found to be a common edible species collected and consumed in plenty by the inhabitants of those areas.

The fungus was identified as *Gomphus floccosus* (Schw.) Singh, (figures 1–3) with the following diagnostic characteristics.

Pileus 4.5–17.5 cm broad, infundibuliform, depressed deeply, margin often undulate, sometimes with overlapping lobes, surface moist to glutinous, innately scaly at the margin to coarsely scaly at

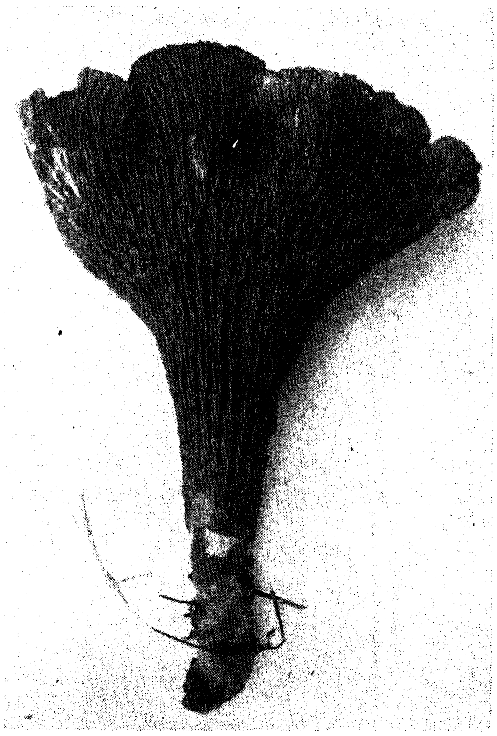
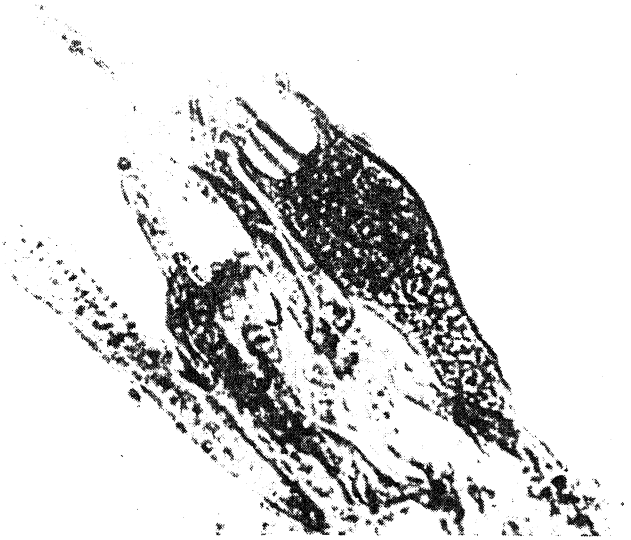


Figure 1. Sporophore of *Gomphus floccosus* (Schw.)

2



3

Figures 2 and 3. Photomicrographs of *G. floccosus* (× 1750): **2,** basidium; **3,** spores.

centre. Colour orange-yellow to orange or reddish orange but paler with age, often more yellow to ochraceous. Context thin to moderately thick, fibrous or brittle. Hymenophore decurrent, often with anastomosed ridges, sometimes extending throughout the basidiocarp length. Spores 11.5–14.5 × 7.8 μm, ellipsoid, ovoid or broadly sub-fusoid, cyanophilous, print ochraceous yellow. Basidia 64.0–100 × 7.5–10.5 μm, tetrasporic. Stipe short, tapering, solid, interior whitish to pale buff, becoming hollow, concolorous with hymenium; flesh white with pleasant aroma. Clamp connections absent.

The fungus shows close resemblance to the species reported from New England[1] and differs appreciably from *G. clavatus*[2] from Gulmarg, Kashmir, India. Also, *G. floccosus* has not earlier been reported from India[3].

The authors are thankful to Dr D. N. Pegler, Royal Botanic Garden, Kew, England, for confirming the identification of the fungus and to the ICAR, New Delhi, for financial assistance.

31 March 1989; Revised 6 September 1989

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OCCURRENCE OF VESICULAR-ARBUSCULAR MYCORRHIZA IN *SUAEDA MARITIMA* (L.) DUMORT—A PIONEER MANGROVE OF THE CHENOPODIACEAE

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MANGROVE forest is an unique ecosystem of plants belonging to several families and adapted to physiological stress due to salinity and waterlogging. The presence of mycorrhiza may be of significance in the plants' adaptive behaviour. Reports on association of vesicular-arbuscular mycorrhiza (VAM) with mangrove roots, specially *Suaeda maritima* (Chenopodiaceae), the primary colonizer of salt marshes, are conflicting. Mason[1] reported the presence of mycorrhizal elements in roots of mangroves, excluding *Suaeda*. Kleca and Vukolov[2] reported the presence of VAM in *Suaeda* roots, which, however, were subsequently found to be either non-mycorrhizal or only very poorly mycorrhizal[3]. Recently, Mohankumar and Mahadevan[4] failed to find VAM in *S. maritima* growing in Pitchavaram mangrove forest in Tamil Nadu, India. We report here the presence of VAM in the roots of *S. maritima* (L.) Dumort growing in the terminal, seabound Gangetic delta in West Bengal, India.

The terminal part of the world's largest delta, known as Sundarban (partly in Bangladesh), criss-crossed by tidal rivers, creeks and channels, is inhabited by about 40 species of mangroves at various stages of succession. *S. maritima* appears as a primary colonizer of riverbed marshes and ridges. The soils are alkaline (pH 7.5 to 8.8), with high soluble salinity (6.9 to 18.5 mmho/cm). The clayey soils have high organic carbon (0.528%) but low nitrogen (0.09%) and low available phosphorus (11.24 ppm). Our search by the usual staining method[5] revealed the presence of typical vesicles in the roots of *S. maritima*. Average root infection intensity of plants collected from five locations varied from 60 to 70% in number infected and 40 to 50% in length of infected roots. Average VAM spore count by wet sieving and decanting method[6] was 92 per 100 g rhizosphere soil. Four species of VAM fungi, viz. *Glomus mosseae* (Nicol and Gerd.) Gerdemann and Trappe, *G. fasciculatum* (Thaxter Sensu Gerd.) Gerdemann and Trappe, *G. multicaulis* Gerdemann & Bakshi and *Gigaspora margarita* Becker & Hall, were present in the rhizosphere soil samples. Only vesicles and connecting hyphae were found in the infected root cortex; arbuscles were not formed.

The Sundarban mangrove forest ecosystem is different from most other mangrove forests of the northern and southern hemisphere. The deltaic land mass of the Sundarban is essentially alluvial in origin. Geological and archaeological evidence[7] show that part of the land mass was strongly mesophytic in recent geological age. Reduction in freshwater flow of upstream rivers and tidal inundation of the land through creeks and channels resulted in formation of hydromorphic saline swamps where mangroves now predominate. This would explain the presence of VAM in the Sundarban ecosystem in contrast to its absence in Pitchavaram[4] and other mangrove forests, which are essentially marine in origin. Herbaceous members of the Chenopodiaceae commonly, but not invariably, lack VAM[8]. The presence of VAM in a Chenopodiaceae species under physiological stress, as seen here and previously[9], suggests that VAM are ecologically significant to plants growing under stress.

The authors thank the Department of Environment, New Delhi, for financial help.

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CYTOLOGICAL STUDY OF BASIDIOSPORE OF *PUCCINIA RUELLIAE* (BERK. & BR.) LAGERH.

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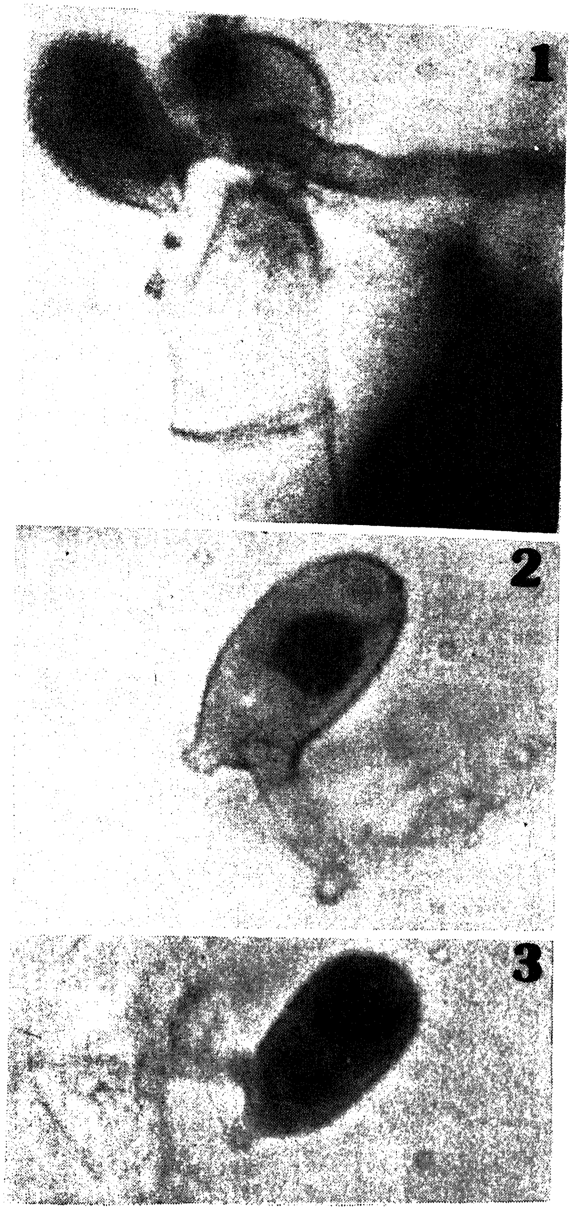
TELIOSPORES of *Puccinia ruelliae* (Berk. and Br.) Lagerh. were reported to produce basidium and basidiospore in tap-water[1]. Development of infection structure by its uredospores[2] and nuclear behaviour during infection structure development[3] have also been studied. This paper presents results of a cytological study of basidiospore of *P. ruelliae*.

Teliospores were scraped from freshly collected leaves of *Ruellia prostrata* Lamk. and allowed to germinate in tap-water on glass slides at 20°C following De and Roy[1]. Teliospores germinated and produced basidiospores within 48 h. The water on the slides was allowed to dry and the teliospores showing development of basidium and basidiospores were fixed in a mixture of equal parts of propionic acid and ethyl alcohol for 24 h. The slides were then washed in 70% alcohol for 5 min, transferred to alcoholic–HCl–carmine stain solution for 24 h at 60°C, rinsed in 70% alcohol and mounted in 45% propionic acid[3].

The teliospores produced 4-celled basidium and each cell developed a basidiospore on a sterigma. Basidiospores were hyaline, thin-walled, smooth, uninucleate, 14.0–17.5 × 5.0–7.0 μm, with broad apiculus. The nucleus within the basidiospore of *P. ruelliae* was beaded, spherical and large, up to 2.0 μm in diameter. No nucleolus was observed. The basidiospores germinated *in situ* producing a single germ tube, and germination took place before the spores were discharged (figure 1) or delayed until after discharge. All the germinating spores observed were uninucleate even after formation of a long germ tube (figures 1–3).

Singh[4] showed formation of binucleate basidiospore of *P. ruelliae* as a result of a mitotic division. Mims[5] recorded uninucleate, binucleate and quadrinucleate basidiospores in *Gymnosporangium juniperi-virginianae* Schw., which inspired me to verify Singh's[4] observation. I observed 1217 basidiospores of *P. ruelliae* but none of them was binucleate or quadrinucleate. Mitosis did not occur in any spore since all the germinating spores observed were uninucleate even after formation of a long germ tube. The present observation thus contradicts that of Singh[4].



Figures 1–3. Germinated uninucleate basidiospores of *Puccinia ruelliae* (Berk. and Br.) Lagerh. **1**, before discharge; **2** and **3**, after discharge.

From a study based on a large number of rust fungi, Anikster[6] concluded that binucleate basidiospores are the general rule in them. This opinion of Anikster[6] is not applicable to *P. ruellae* because the present study reveals that its basidiospores are not binucleate but uninucleate. However, the nucleus within the basidiospores of *P. ruelliae* was beaded and the beaded appearance was probably due to its heterochromatic nature.

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SOME NEW REPORTS ON KERATINOPHILIC FUNGI

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THE isolation of keratinophilic fungi and related dermatophytes by the hair baiting method has revealed their occurrence in the soil of various habitats of India and other locations[1-5]. Reports on the occurrence of keratinophilic fungi in stress habitats such as house dust are few. The present study provides information on the occurrence of keratinophilic fungi in house dust in India for the first time. The keratinophilic fungi and dermatophytes were isolated by hair baiting method[6, 7] and cultures were maintained on Sabouraud's dextrose agar at 30±2°C. Some of the fungi were also deposited at ITCC, New Delhi; IMI, Kew; and UAMH, Alberta.

Of the 91 house dust samples collected from 13 sites of Kanpur, India, 84.3% samples yielded 35 taxa, listed below in decreasing occurrence (%): *Chrysosporium carmichaeli* (100), *Chrysosporium farinicola* (100), *Myceliophthora* anamorph of *Corynascus novoguineensis* (100), *Chrysosporium tropicum* (100), *Arthroderma flavescens* (62.1), *Chrysosporium tuberculatum* (29.2), *Aphanoascus* species (19.5), *Chrysosporium* species (15.4), *Trichophyton flavescens* (14.4), *Chrysosporium keratinophilum* (14.2), *Chrysosporium merdarium* (14.2), *Chrysosporium queenslandicum* (14.2), *Microsporum* species (11.1), *Microsporum fulvum* (11.1), *Microsporum gypseum* (11.1), *Trichophyton vanbreuseghemii* (9.7), *Arthroderma gertleri* (7.3), *Arthroderma teereus* (7.3), *Chrysosporium crassitunicatum* (7.0), *Chrysosporium sulphureum* (4.8), *Chrysosporum indicum* (2.5), *Chrysosporium pannicola* (2.5), *Chrysosporium lucknowense* (2.4), *Myceliophthora* anamorph of *Arthroderma tuberculatum* (2.4), *Myxotrichum* (2.4), *Onygena* (2.4), *Ctenomyces serratus* (2.2), *Chrysosporium evolceanui* (1.0), *Acremonium obclavatum* (1.0).

This work was financed by CSIR, New Delhi. We thank Drs L. Ajello and A. A. Padhye, Division of Mycotic Diseases, Centre for Infectious Diseases, Atlanta, USA for confirming identification of some of the fungi, and Dr R. A. Samson, Central Bureau voor Schimmelcultures, Baarn, The Netherlands, for identification of *Acremonium obclavatum*.

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EPIDERMAL SURFACE PATTERNS OF ACHENE IN *ELEOCHARIS* R. BR. (CYPERACEAE)

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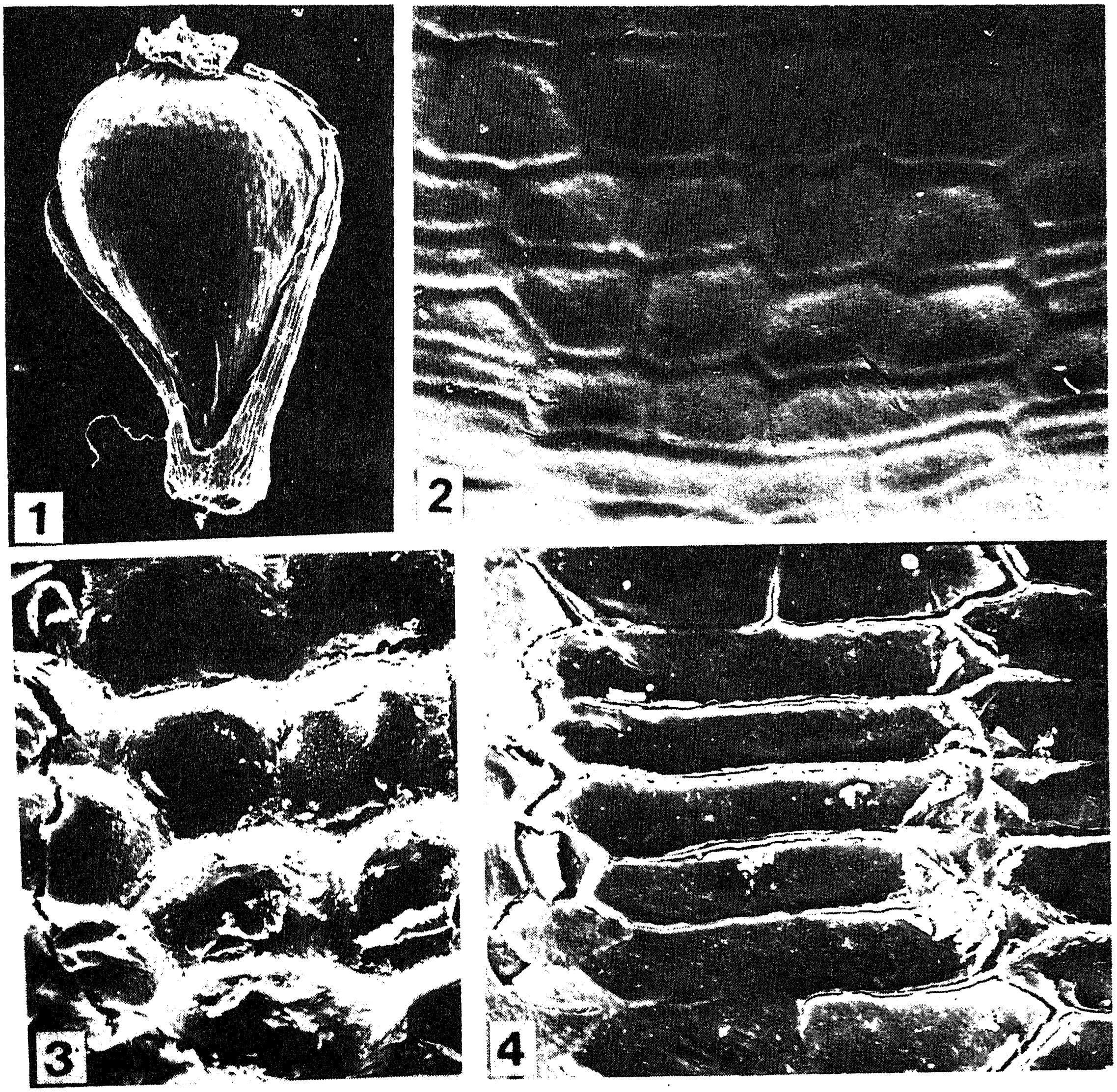
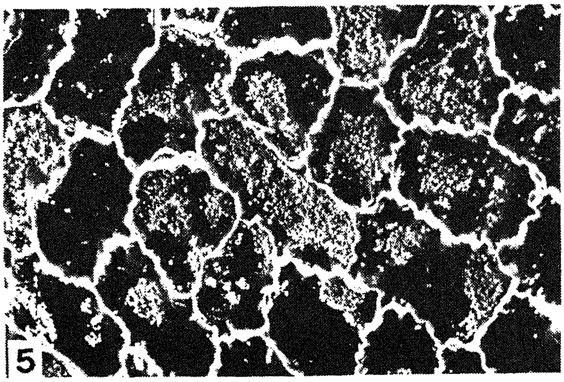
IN the Cyperaceae, scanning electron microscopic studies of achene surfaces provide criteria useful for the delimitation of taxa at various levels[1,2]. A perusal of the literature reveals that no work has been done on achene micromorphology in the genus *Eleocharis*. The present work was on *E. acutangula*. (Roxb.) Schult, *E. atropurpurea* (Retz.) Presl., *E. congesta* D. Don., *E. dulcis* (Burm. f.) Henschel, *E. palustris* (L.) R. & S. and *E. retroflexa* (Poir) Urb.

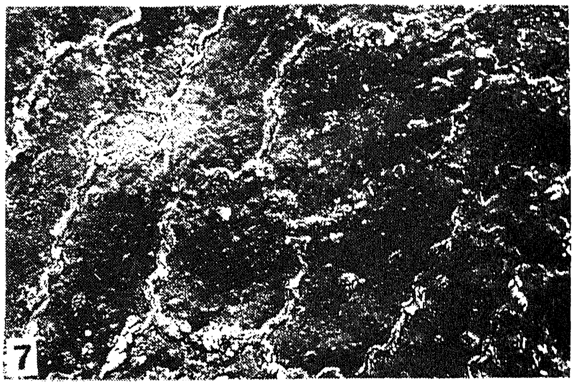
The genus *Eleocharis* of the tribe Cypereae (subfamily Cyperioideae, family Cyperaceae) includes 150 species distributed from the tropics to the polar regions of both hemispheres, with the greatest concentration in the New World[3]. In India, the genus is represented by 16 species[4] and in Bihar state by 7 species[5]. The genus is mainly characterized by persistent 5–8 bristles, and dilated style base articulated with ovary and persistent on the fruit (figure 1).

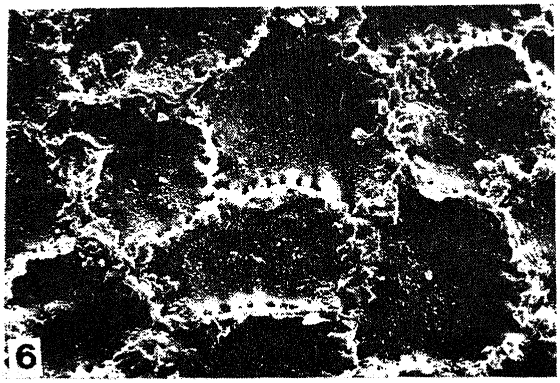
Achenes of *Eleocharis* species were taken from herbarium specimens collected by the authors from different localities of eastern Bihar. Voucher specimens are deposited in the Bhagalpur University Herbarium. Achenes were subjected to cleaning by sonication, followed by soaking for about 18–36 h in 1:9 concentrated sulphuric acid : acetic anhydride solution to remove the outer walls of epidermal cells. They were then rinsed in distilled water and desiccated prior to sputter coating with gold (200 Å).



Figures 1–4. Scanning electron micrographs of **1**, fruit of *Eleocharis atropurpurea* (× 94); and achene epidermal cell surface of **2**, *E. atropurpurea* (× 1000); **3**, *E. retroflexa* (× 400); and **4**, *E. acutangula* (× 300).







Figures 5–7. Scanning electron micrographs of achene epidermal cell surface of **5**, *E. palustris* (× 1000); **6**, *E. dulcis* (× 1000); and **7**, *E. congesta* (× 2000).

Both treated and untreated achenes were examined under a JEOL-JSM 35 C scanning electron microscope at the National Botanical Research Institute, Lucknow.

In *E. atropurpurea*, the epidermal cells of the achenes are small and show 4–8 anticlinal walls. The epidermal cells show polygonal outline and have more or less straight walls (figure 2). The epidermal cells in *E. retroflexa* are small and show broad anticlinal walls (figure 3). In *E. acutangula* epidermal cells are broader than long and are arranged in a parallel fashion (figure 4); the anticlinal walls are straight and thin. *E. palustris* shows epidermal cells with wavy and moderately thick anticlinal walls (figure 5). Epidermal cells in *E. dulcis* show polygonal outline (figure 6); the anticlinal walls are prominent and show characteristic perforations. *E. congesta* has epidermal cells with irregular outline (figure 7); the anticlinal walls are less prominent.

The epidermal patterns of *Eleocharis* achene surfaces can be used to distinguish the taxa as each species has its own characteristic ornamentation. Based on achene surface pattern, *E. palustris* (series Palustriformes) and *E. congesta* (series Multicaules) show close similarity. *E. acutangula* and *E. dulcis*, placed together in series Mutatae[6], show entirely different patterns (figures 4 and 6). *E. atropurpurea* (series Maculosae) and *E. retroflexa* (series Tenuissimae) remain distinctive from the other *Eleocharis* species studied presently. To elucidate the generic relationships of *Eleocharis*, a detailed study of achene micromorphological characters in other related genera is in progress.

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RELATIONSHIP BETWEEN PHOSPHATASE ACTIVITY AND SEX EXPRESSION IN *COCCINIA INDICA* W. & A.

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THE formation of stamen and ovary are the result of two different embryological events leading to the differentiation of male and female reproductive organs[1]. Plant growth regulators have been shown to regulate sex organ differentiations[2-4]. The activities of several enzymes seem to be affected by exogenous growth regulators[5,6]. Studies have been carried out to establish a correlation between enzyme activities and sex expression[6-8]. This report describes the patterns of acid and alkaline phosphatase distribution in vegetative and reproductive parts of male and female plants of *Coccinia indica*.

For enzyme analysis, vegetatively growing shoot tips, flower buds (5 mm in size) and mature flowers (at anthesis) were harvested from male and female plants of *C. indica* growing in the campus of the university. Known amounts of different tissues were homogenized in 0.1 M Tris–HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol[9] using a chilled pestle and mortar. The homogenates were centrifuged at 12,000 rpm for 20 min at 0–4°C. The clear supernatants were used as enzyme source.

Acid and alkaline phosphatase activities were determined at pH 4.8 and 9.0 respectively disodium *p*-nitrophenylphosphate as substrate. The total and specific activity of each enzyme were expressed as units per gram fresh weight and units per milligram protein respectively. The units represent μg of *p*-nitrophenol produced per minute at 30°C. Protein was estimated colorimetrically by the procedure of Lowry *et al.*[10] using bovine serum albumin as standard. The experiments were repeated twice.

Acid phosphatase isoenzymes were separated electrophoretically on 7.5% polyacrylamide slab gels (1 mm thick). After electrophoresis, the gels were rinsed for about 30 min in sodium acetate buffer (pH 4.8). The buffer was changed every 10 min to lower the pH of the gel from 9.0 to 4.8. The gel was then transferred to a reaction mixture containing α-naphthyl phosphate and Fast Garnet GBC salt, and incubated at 30°C until brown bands appeared[11].

Changes in acid phosphatase and alkaline phosphatase activity in male and female plants/flowers were recorded (table 1). Marked differences in both total and specific acid phosphatase activity were observed between reproductive tissues of male and female plants. Male flower buds and mature flowers had significantly higher levels of acid phosphatase activity compared to female flowers. Total and specific activity of the enzyme increased in developing male reproductive organs. Specific acid phosphatase activity increased in developing female flowers also, but the total activity in female tissues was less than in vegetative tissues. Acid phosphatase activity in vegetative shoot tips of male and female plants were similar.

The highest alkaline phosphatase activity was recorded in male mature flowers and the lowest in female mature flowers (table 1). There was no marked difference in alkaline phosphatase activity between male and female vegetative tissues.

Three isoenzymes of acid phosphatase, designated A, B and C, were observed in vegetative shoot tips of

Table 1 *Acid and alkaline phosphatase activity in vegetative and reproductive tissues of male and female plants of Coccinia indica*

		Acid phosphatase		Alkaline phosphatase	
Sex	Developmental stage	(Units/g fr. wt)	(Units/mg protein)	(Units/g fr. wt)	(Units/mg protein)
Male	Vegetative shoot tips	41.0 ± 1.8	0.49 ± 0.02	10.0 ± 0.9	0.12 ± 0.01
	Flower buds	43.5 ± 1.8	0.91 ± 0.04	11.2 ± 0.2	0.23 ± 0.01
	Mature flowers	45.5 ± 4.2	1.20 ± 0.11	11.5 ± 0.7	0.29 ± 0.05
Female	Vegetative shoot tips	43.2 ± 1.3	0.48 ± 0.02	10.5 ± 1.1	0.12 ± 0.01
	Flower buds	33.0 ± 2.3	0.56 ± 0.04	3.3 ± 0.7	0.06 ± 0.01
	Mature flowers	33.3 ± 1.8	0.61 ± 0.03	2.8 ± 0.6	0.05 ± 0.01

Data represent mean of 3 replicates ± S.D.

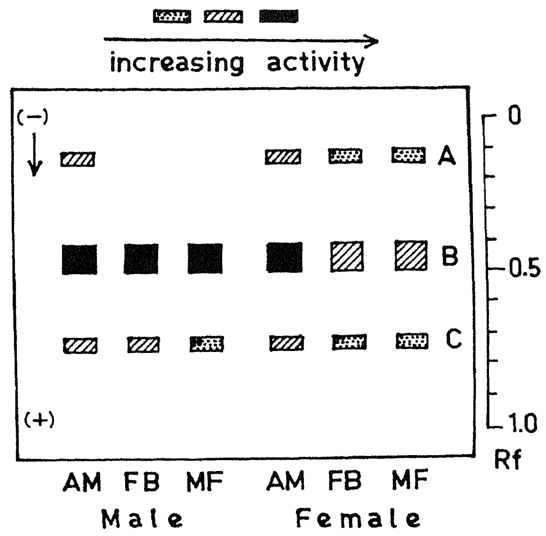

Figure 1. Zymograms showing the patterns of acid phosphatase (pH 4.8) isoenzyme distribution in vegetative and reproductive tissues of male and female plants of *Coccinia indica*. [AM, vegetative shoot tips; FB, flower buds; MF, mature flowers.]

both male and female plants, each with similar relative electrophoretic mobility and intensity in both sexes (figure 1). However, band A was absent in developing and mature male flowers, and the intensity of band C decreased during flower development. All three bands were present in female reproductive tissues but bands A and C were less intense in flowers than in vegetative tissues.

Gibberellins are known to favour male sex expression in many plants, including cucurbitaceous species. A correlation between higher gibberellin content and male sex expression has been established[4,12]. It has been demonstrated that gibberellin treatment enhances acid phosphatase activity and its secretion in plant tissues[5,13]. It appears that acid and alkaline phosphatase activities are enhanced in male reproductive tissues as a result of higher endogenous gibberellin content of these tissues, as suggested earlier[8].

Changes in isoenzyme patterns during organ differentiation and growth have been reported in several plants[14]. The present results suggest that acid phosphatase isoenzyme A, present in vegetative tissues, disappears in male reproductive organs during their formation but not in female reproductive organs. It is likely that repression of certain gene(s), specific to acid phosphatase isoenzyme A, is responsible for male sex expression, whereas female sex expression is favoured by the continued expression of such gene(s) in *C. indica*.

One of the authors (ML) thanks CSIR, New Delhi, for a fellowship.

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INDUCTION OF MUTATION THROUGH INTERSPECIFIC GRAFTING

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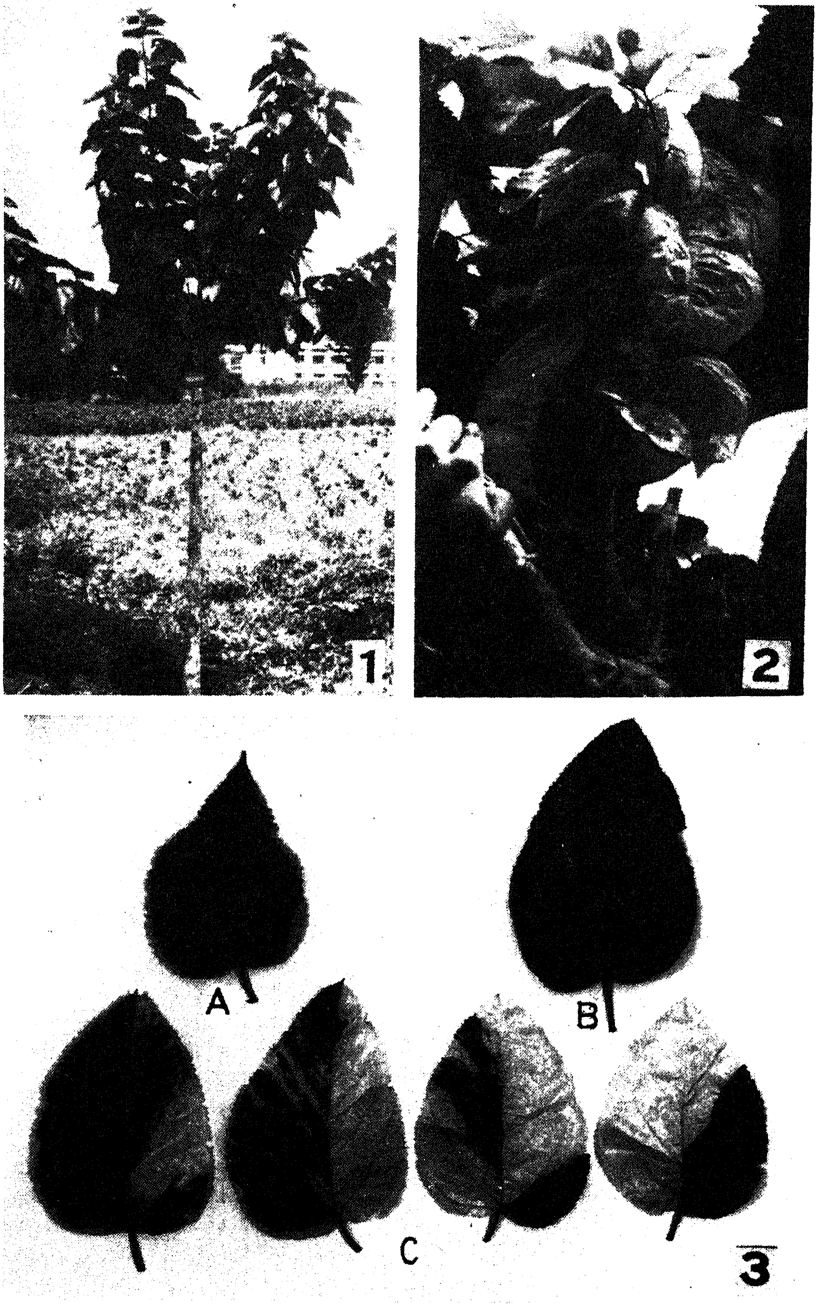
PHYSICAL and chemical mutagens are the major agents used to induce mutations. It has also been reported that sudden damage to apical portions of plants, severe attack of pests and interspecific/intraspecific grafting resulted in some heritable changes in the plants. Change of sex from male to female by decapitation in papaya has been noticed by earlier workers. Breaking of apical dominance by pinching



Figures 1–3. **1**, Branches from grafted buds. **2**, Variegated branch. **3**, Variegation pattern, A, leaf of the stock; B, leaf of the scion; C, leaves of the variegated scion.

has also affected sex expression in mulberry[1]. Similarly interspecific grafting has been employed to change the sex of dioecious plants. Often, to change the sex of a particular strain, it is grafted on a stock bearing the desired sex.

It is observed that when interspecific/intraspecific grafting is done with different scions and stocks, sometimes the scion gets some of the features of the stock. Change of sex in scion has been reported[2] in mulberry through grafting of strains of different sex.

This note reports occurrence of a variegated mutant in mulberry when axillary buds from exotic strains were grafted on to branches of a local strain. To establish a mulberry germplasm of temperate strains, which are difficult to root, bud grafting of exotic strains on the established local trees was undertaken. Nearly 60 varieties were grafted on local trees. Among them 18 buds of Kokuso-21, an improved Japanese mulberry variety of *M. multicaulis*, were grafted on a *M. indica* tree of 6–7 years (figure 1).

A week after grafting, the top portion above the grafted bud was pruned to facilitate quick sprouting of scion buds. When the buds sprouted, among 12 sprouts one was found producing variegated leaves. This branch was allowed to grow till December of the same year. It attained a growth of 127 cm with 55 leaves. All the leaves were variously variegated (figure 2). The variegation pattern was irregular (figure 3). The mutated scion bears features present in neither the stock nor the scion. The leaves are also thin and differ in size from those of both the parents. The length of the stock branch was 242 cm, with 22 leaves per metre and internodal distance of 4.5 cm; the girth of the branch was 1.5 cm; the area of the leaf was 638 cm^2. The length of nonmutated scion was 218 cm, with 30 leaves per metre and internodal distance of 3.3 cm; the girth of the branch was 2 cm; the area of the leaf was 231.25 cm^2. The length of the mutant branch was 127 cm, with 43 leaves per metre and internodal distance of 2.3 cm; the girth of the branch was 1 cm; the area of the leaf was 187 cm^2.

From the above, it is clear that the length of the mutant branch, internodal distance and leaf area are reduced when compared to those of the nonmutant scion and stock.

The mutant is being multiplied and observations being made to assess its value to sericulture. The variegated nature of the leaves can serve as a marker in genetic studies. In addition, the variegated leaves can be of horticultural importance.

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CADMIUM INDUCIBLE PROTEINS IN *SCENEDESMUS QUADRICAUDA*

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HEAVY metals like cadmium, lead, mercury, copper, etc. are increasing in the biosphere through pollution, agricultural runoff and mining activities. They have direct bearing on various physiological and biochemical functions of organisms. The pronounced effects of heavy metal toxicity are reduction in growth and photosynthesis, inhibition of enzyme activities, and degradation of chloroplasts and mitochondria. Heavy metal tolerance in plants has been explained by several views: exclusion from plant parts[1], accumulation of metals in vacuoles[2] and on cell walls[3], evolution of metal-tolerant enzymes[4], and induction of specific metal-binding proteins similar to metallothioneins[5]. Metals like cadmium, zinc, lead, silver, antimony, copper, mercury, gold, beryllium, tin and nickel induce these proteins[6], which are thought to be involved in metal ion homeostasis and many other functions, such as regulation of cellular metabolism, control of cellular growth, detoxification of free radicals and excess metal ions, and protection against ionizing radiation[7].

Algae and cyanobacteria were studied for metal toxicity[8]. The first report of cadmium-inducible binding proteins was in the freshwater blue-green alga *Anacystis nidulans*[9]. Metal-inducible proteins were also reported in some other algae, viz. *Chlorella ellipsoida*[10], *C. pyrinoidosa*[11], *Dunaliella bioculata*[12] and *Synechococcus* sp.[13]

The present report deals with cadmium-inducible proteins in the common freshwater alga *Scenedesmus quadricauda*. *S. quadricauda* cells were grown in modified Chu medium[14]. Cultures were maintained at 25±2°C in a 16 h light and 8 h dark cycle. Cultures were exposed to different concentrations of cadmium chloride (1, 10, 50, 100, 200 and 400 μM Cd) and growth was measured as increase in optical density at 678 nm and chlorophyll *a*. Figure 1A shows growth curves of unexposed and cadmium-exposed cells. Growth (as chl *a*) was increased at low concentration of cadmium (1 μM), but inhibited about 18% in 10 μM, 63% in 50 μM, 68% in 100 μM, 90% in 200 μM and totally in 400 μM cadmium (figure 1B). Cadmium causes inhibition of chlorophyll synthesis, decreases the chl *a*/*b* ratio, and causes disorganization of grana[15]. We also found decreased synthesis of chl *a* and chl *b* in cadmium-exposed cells (table 1). At 100 μM cadmium chl *a* and chl *b* were decreased by about the same extent. However, at higher concentration (200 μM), chl *b* synthesis was inhibited to a greater extent.

Cadmium-exposed (200 μM) and unexposed cells were collected by centrifugation (6000 *g*, 10 min), subjected to sonication in 0.5 M Tris–HCl buffer (pH 8.6), and centrifuged at 4°C (12,000 *g*, 10 min). The supernatant was acetone-precipitated and electrophoresis was done on an 18% SDS-polyacrylamide gel[16]. Cadmium-exposed cells showed an additional band co-migrating with a molecular mass marker of 8 kDa (figure 2). This band was not observed in unexposed cells. This protein may be similar to metal-binding proteins reported from other algae. Cadmium-binding protein from *Synechococcus* sp. has a molecular mass of 8.1 kDa, whereas in *Dunaliella* it is 10 kDa. The molecular mass of purified metal-binding proteins from different algae ranges from 8 to 10 kDa.

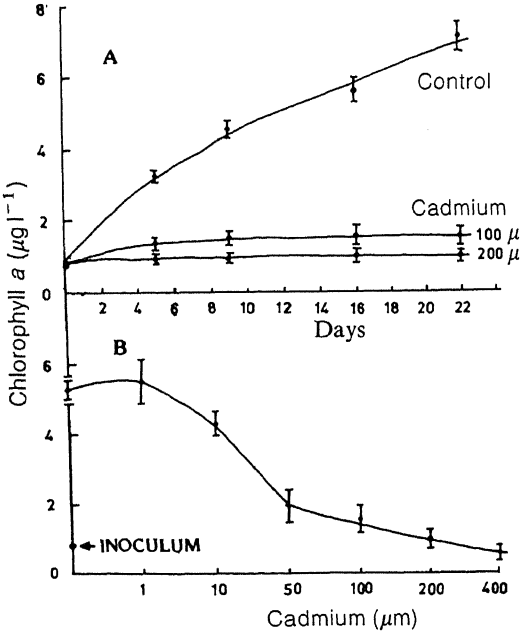

Figure 1. **A**, Growth curves (as chl *a*) of cadmium-exposed and unexposed cells of *Scenedesmus quadricauda*. **B**, Effect of different concentrations of cadmium on *S. quadricauda*.

Table 1 *Effect of cadmium on chl a and chl b synthesis in S. quadricauda*

Cadmium (μM)	Chl *a*	Chl *b*
None (control)	4.54 ± 0.54	1.46 ± 0.23
100	1.52 ± 0.42	0.58 ± 0.17
200	1.00 ± 0.3	0.27 ± 0.12

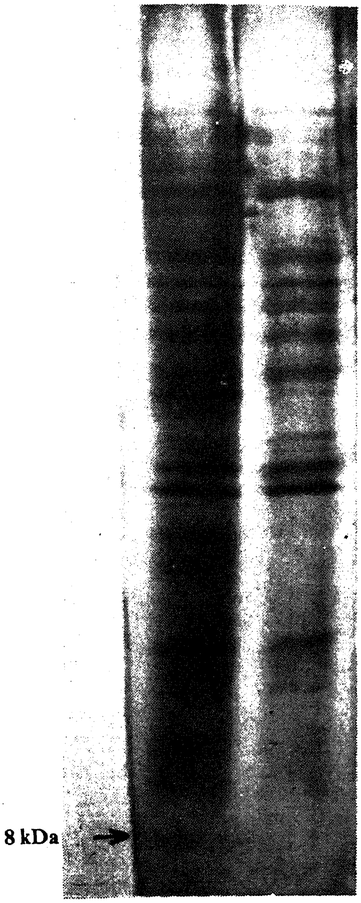

Figure 2. Electrophoretic protein profile of cadmium exposed (left) and unexposed (right) *S. quadricauda*.

The reported cadmium-inducible protein (8 kDa) in *S. quadricauda* is presumed to be a cadmium-binding protein and hence can be considered as an environmental indicator of cadmium pollution. Further work on purification and metal-binding properties of this protein is in progress.

One of the authors (GNR) is grateful to CSIR, New Delhi, for a fellowship.

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Note added in proof: Metal binding proteins in algae now reported to be poly(γ-glutamylcysteinyl) glycines as in higher plants (see Gekler *et al.*, *Arch. Microbiol.*, 1988, **150**, 197).

REGENERATION OF SHOOT BUDS FROM CALLUS CULTURES OF *PAPAVER SOMNIFERUM*

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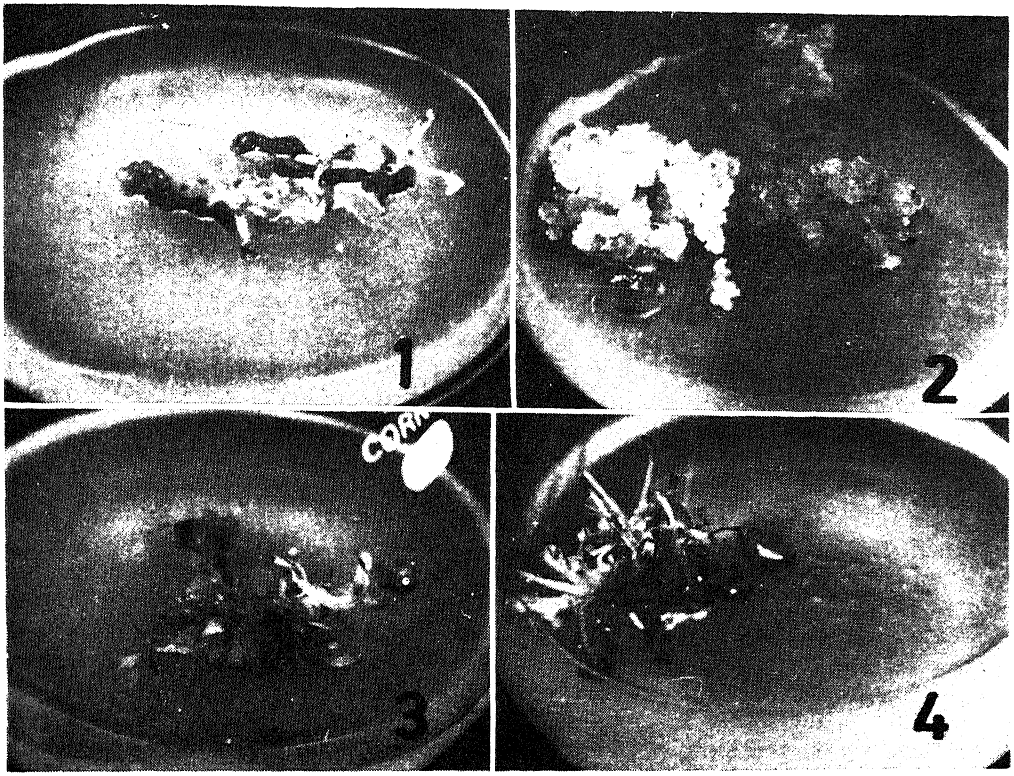
TISSUES from opium poppy have been grown in culture as a callus[1-3], but most of these studies were aimed at studying alkaloid metabolism. In the present investigation, efforts have been made to culture various explants from the opium poppy, *Papaver somniferum* L., and induce organogenesis *in vitro*.

Hypocotyl segments, cotyledons, seedling roots, stem segments and leaf discs of *P. somniferum* were inoculated onto Murashige and Skoog[4] (MS) basal medium supplemented with sucrose (3%) and various concentrations (0.05–5.0 mg/l) of kinetin or benzyladenine singly or in combination with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthalene-acetic acid. Callus initiation was observed only from seedling root explants on MS medium containing 0.5–1.0 mg/l of kinetin and 0.5–3.0 mg/l of IAA (figure 1). Other combinations of growth regulators failed to evoke any response in any of the other explants. The callus initiated on medium containing 0.5 mg/l of kinetin and 0.5 mg/l of IAA was isolated and maintained as stock callus on medium supplemented with 0.5 mg/l of kinetin and 3.0 mg/l of 2,4-D (figure 2). This callus was creamy-white, friable and actively growing, and was subcultured regularly every 30th day. The stock callus in its 5th passage of subculture was transferred onto medium containing kinetin or benzyladenine singly or in combination with auxins for differentiation. Shoot bud regeneration was observed only on medium containing kinetin (0.05–1.0 mg/l) alone or kinetin (0.05–3.0 mg/l in combination with 0.5 mg/l of IAA (table 1). Other growth regulators singly or in combinations did not evoke any response. Shoot buds (2–5 in number) were regenerated on MS medium containing 0.05–1.0 mg/l of kinetin. Further increase in kinetin concentration (3.0–5.0 mg/l) inhibited shoot bud regeneration completely. Addition of IAA (0.5 mg/l) to medium containing 0.05–3.0 mg/l of kinetin improved the shoot bud regeneration response of the callus. The maximum number of shoot buds (6–8) was obtained



Figures 1–4. Tissue culture of opium poppy. **1,** Callus initiation from seedling root on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l). **2,** Stock callus (30 days old) on MS + kinetin (0.5 mg/l) + 2,4-D (3.0 mg/l). **3,** Shoot buds regenerated on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l). **4,** Isolated shoot buds on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l).

Table 1 *Effect of various concentrations of kinetin singly or in combination with IAA on regeneration of shoot buds in callus cultures of P. somniferum*

	Response				
	IAA in mg/l				
Kinetin (mg/l)	0	0.5	1.0	3.0	5.0
0.05	S (2–3)	S (4–6)	NR	NR	NR
0.5	S (2–5)	S (6–8)	NR	NR	NR
1.0	S (2–3)	S (4–5)	NR	NR	NR
3.0	NR	S (2–5)	NR	NR	NR
5.0	NR	NR	NR	NR	NR

S, Shoot buds; NR, no response. Numbers in parentheses give numerical range of shoot buds per five flasks.

on medium containing 0.5 mg/l of kinetin and 0.5 mg/l of IAA (figure 3). This complete elimination/replacement of 2,4-D with IAA favoured shoot bud regeneration. These results indicate the inhibitory effect of 2,4-D on shoot bud regeneration in *P. somniferum*, as observed in cereals[5].

Shoot buds differentiated on these media were 4–5 mm long. They were isolated and transferred into the same medium or other media containing various combinations of auxins, cytokinins and gibberellic acid for elongation and/or rooting. Shoot buds elongated up to 15 mm on media containing 0.5–1.0 mg/l of IAA and 0.05–3.0 mg/l of kinetin (figure 4). Gibberellic acid did not cause elongation of shoot buds. Under no circumstances could rooting be induced in isolated shoot buds. Further experiments are in progress to induce rooting and to obtain complete plantlets.

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KARYOTYPE ANALYSIS OF *PAPAVER DUBIUM* L.

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PAPAVER DUBIUM L. is an important medicinal plant of the family Papaveraceae. The latex from its immature capsules is known to possess two alkaloids, viz. aporein and aporeidin. Aporein has a physiological action resembling thebaine. The petals of *P. dubium* are sudorific and contain cyanidin-B and pelargonidin-C (ref. 1). The chromosome numbers reported[2] in this species are $2n=28$ and $2n=42$ (ref. 2). However, in a literature survey we could not find any report on chromosome morphology of *P. dubium*. The karyotype of this medicinally important plant species is described in this communication.

Actively growing roots (1–2 cm long) originating from germinated seeds were fixed in Carnoy's solution (6 parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) after pretreatment with saturated solution of *p*-dichlorobenzene at 15°C for 3 h, and transferred to 70% alcohol. After fixation, they were hydrolysed in 1 N HCl at 60°C for 5–10 min and then stained and squashed in 2% acetocarmine. Photographs of ten well-spread metaphase plates were made using phase contrast optics on an Olympus Vanox-S microscope from temporary slides and used for analysis. The chromosomes of the haploid complement were numbered 1 to 14 in decreasing order of length. Centromere positions were described following the nomenclature of Levan *et al.*[3] The centromeric index was calculated as per cent ratio of short arm length to long arm length.

The morphological characteristics of *P. dubium* chromosomes are presented in table 1. A single metaphase cell and its karyogram are shown in figure 1. The somatic chromosome number of *P. dubium* was found to be $2n=28$, which is in conformity with an earlier report[2]. The haploid complement was measured at 41.46 μm. An easily descernible secondary constriction was associated with the long arm of chromosome 2. The chromosome length varied from 2.45 to 3.52 μm. A narrow range of variation in arm ratio (0.26 to 0.47) clearly indicated that all the 14 chromosomes of the haploid complement are acrocentric with subterminally located centromeres. The centromeric index ranged between 19.05 and 31.40, suggesting that the karyotype is symmetric.

Table 1 *Details of chromosome morphology in Papaver dubium L.*

Chromosome number	Chromosome length (μm)				Arm ratio (s/l)	Centromere position	Centromeric index
	Total	Relative	Short arm	Long arm			
1	3.52 ± 0.03	100.00	1.03 ± 0.02	2.41 ± 0.01	0.43	st	29.26 ± 0.29
2*	3.38 ± 0.02	96.02	0.97 ± 0.01	2.36 ± 0.01	0.41	st	28.69 ± 0.18
3	3.28 ± 0.01	93.18	1.03 ± 0.01	2.17 ± 0.02	0.47	st	31.40 ± 0.20
4	3.21 ± 0.02	91.19	0.97 ± 0.01	2.17 ± 0.01	0.45	st	30.22 ± 0.22
5	3.14 ± 0.03	89.20	0.67 ± 0.02	2.38 ± 0.01	0.28	st	21.34 ± 0.32
6	3.07 ± 0.02	87.22	0.62 ± 0.01	2.41 ± 0.01	0.26	st	20.19 ± 0.20
7	2.97 ± 0.02	84.37	0.67 ± 0.01	2.24 ± 0.01	0.30	st	22.56 ± 0.15
8	2.89 ± 0.02	82.10	0.62 ± 0.01	2.24 ± 0.02	0.28	st	21.45 ± 0.22
9	2.86 ± 0.01	81.25	0.76 ± 0.02	2.07 ± 0.01	0.37	st	26.57 ± 0.24
10	2.79 ± 0.02	79.26	0.72 ± 0.01	2.00 ± 0.01	0.36	st	25.81 ± 0.21
11	2.72 ± 0.02	77.27	0.69 ± 0.02	2.00 ± 0.01	0.35	st	25.37 ± 0.38
12	2.66 ± 0.03	75.57	0.55 ± 0.01	1.96 ± 0.01	0.28	st	20.68 ± 0.54
13	2.52 ± 0.03	71.59	0.48 ± 0.02	1.72 ± 0.02	0.28	st	19.05 ± 0.39
14	2.45 ± 0.03	69.60	0.48 ± 0.01	1.55 ± 0.01	0.31	st	19.59 ± 0.26

st, Subterminal.
*Satellited chromosome.

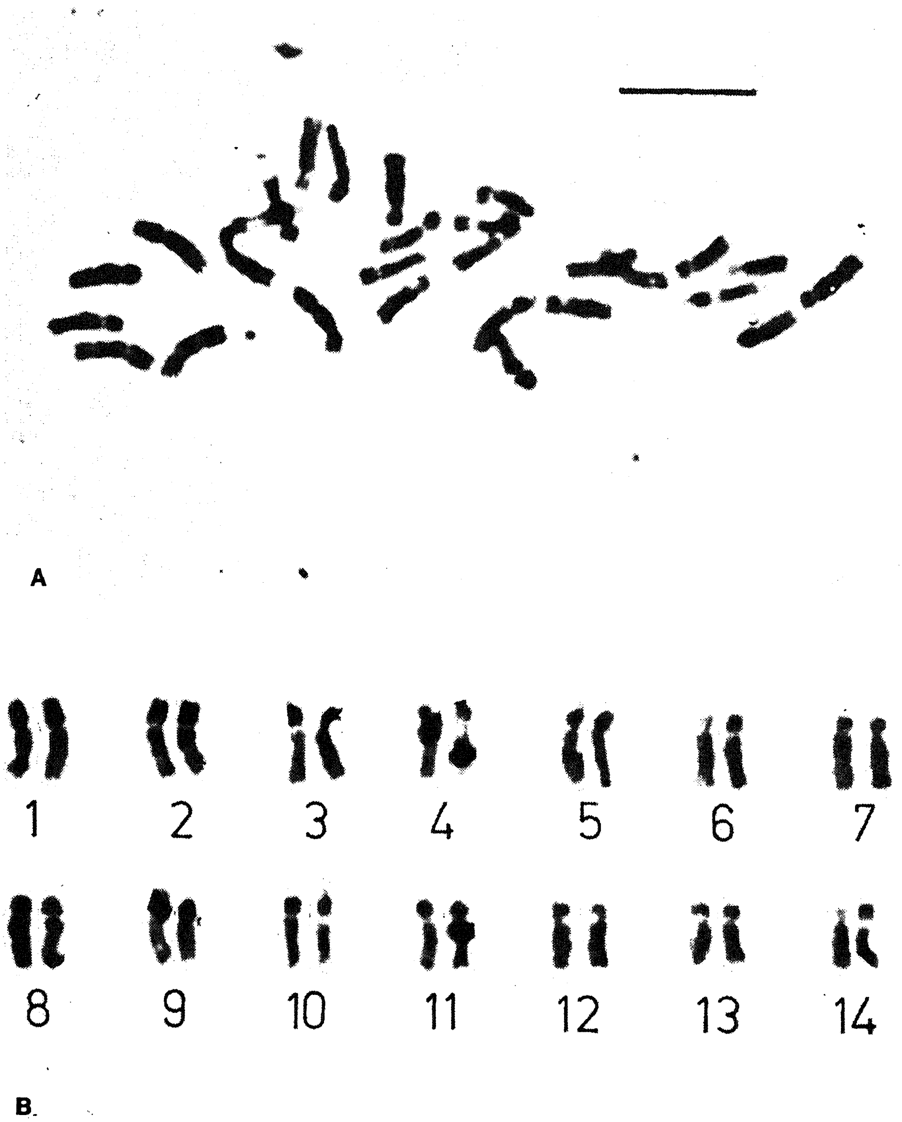

Figure 1A, B. Somatic chromosomes of *Papaver dubium* L. **A,** Metaphase cell showing 28 chromosomes (bar = 10 μm). **B,** Karyogram of the cell in **A**.

16 January 1989; Revised 18 September 1989

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SHOOT-TIP CULTURE IN *DOLICHOS BIFLORUS* L.

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IN VITRO culture of meristems has wide application in crop improvement programmes. It can be used to

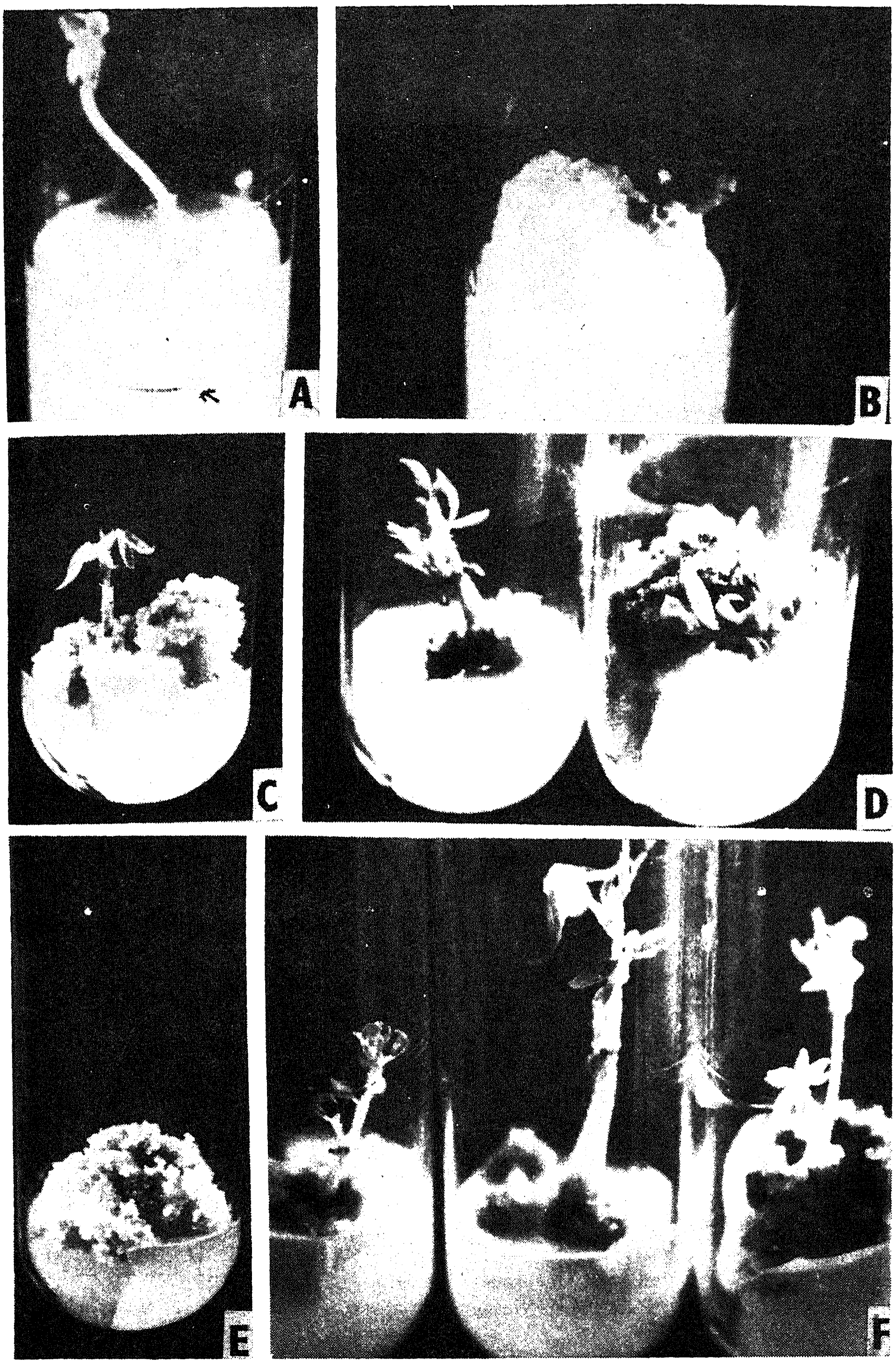

Figure 1A–F.

obtain virus-free material, clonal propagation and conservation of germplasm[1,2]. Legumes play an important role in human and animal diet, providing seeds which are rich in proteins. Most of them are susceptible to various diseases which result in low yield and poor quality of seeds. The seeds, under such conditions, often carry pathogens internally and transmit them to the next generation. In addition, not much success has been achieved in creating significant genetic variability and disease resistance through conventional breeding programmes. Therefore micropropagation techniques have been employed in the improvement of various leguminous crops[3-5]. Although legumes were generally considered as recalcitrant to culture conditions some of the results obtained in recent years are quite encouraging[6,7].

Dolichos biflorus L. (horse gram) is one of the important rainfed legumes used as a pulse, fodder and green manure in several parts of South India. Earlier workers[7,8] have been able to induce callus formation, organogenesis and regeneration of plantlets using cotyledon, hypocotyl, leaf and anther explants, but no attempt was made to culture the shoot tip. The present investigation was undertaken to study shoot-tip morphogenesis in *Dolichos biflorus* L. under *in vitro* conditions.

Seeds were obtained from the University of Agricultural Sciences, Bangalore. They were rinsed in 70% ethanol for 2–3 min and washed several times with sterile distilled water. Surface sterilization was done with freshly prepared chlorine water for 10–15 min, followed by 0.1% mercuric chloride for 3–5 min. The seeds were finally thoroughly washed with sterile distilled water.

Aseptic seedlings were obtained by germinating the sterilized seeds on humidified cotton in culture tubes. Shoot tips about 0.5 to 0.7 cm long were excised from one-week-old dark-grown aseptic seedlings and inoculated on MS medium[9] containing 2% sucrose and 0.8% agar supplemented with naphthleneacetic acid (NAA) and 6-benzylaminopurine (BAP) at various concentrations. The pH of the medium was adjusted to about 5.8 before autoclaving at 15 psi for 15 min. Cultures were maintained at 25 ± 2°C. Two sets of cultures were maintained, one in complete darkness and another under 8:16 h light/dark cycle. The experiments were repeated thrice.

When shoot apices were cultured on MS medium without any growth regulators, they showed growth and rhizogenesis only for a short period (figure 1A). Despite subculturing, the shoot apices ceased to grow and finally shrivelled after four weeks. A similar observation has been reported in *Trapaeolum majus*, *Lupinus albus*[10,11], *Trifolium repens*, *T. pratens*[12,13] and *Vigna unguiculata*[14]. When the medium was supplemented with NAA (2 mg/l), the explants produced vigorously growing calli and rooting, but there was no growth of shoot apices (figure 1B).

With addition of BAP in increasing concentration from 0.1 to 2 mg/l and keeping NAA concentration at 0.1 mg/l, the explants showed excessive callusing at the basal part of the explant in addition to shoot growth and formation of multiple shoots (figure 1C). The latter are more predominant when BAP concentration in the medium is 0.3 and 0.5 mg/l (figure 1D). However, rhizogenesis became completely inhibited in all the combinations. Higher concentrations of BAP (2 mg/l), though favouring profuse callusing (figure 1E), inhibited shoot growth, formation of multiple shoot buds and roots (table 1). Therefore a proper ratio of auxin and cytokinin is essential for growth and regeneration of shoot tips and multiple shoots.

It was noticed that dark incubation is stimulatory to stem elongation and induction of multiple shoot buds (figure 1F). This has been attributed to inhibitory effects on activity of polyphenolic compounds during dark incubation[14], thereby triggering growth and induction of multiple shoot buds. However, the green, nodulated callus obtained from

Figure 1A–F. Shoot-tip cultures in *Dolichos biflorus* L. **A,** Regeneration of shoot and root on MS medium. **B,** Callus on MS medium containing 2 mg/l NAA. **C,** Culture on MS medium containing 0.1 mg/l NAA and 0.1 mg/l BAP showing callusing and shoot growth. **D,** Cultures on MS medium containing 0.1 mg/l NAA + 0.3 mg/l BAP (left) and 1 mg/l NAA + 0.5 mg/l BAP (right) showing shoot growth and multiple shoot buds. **E,** Culture on MS medium containing 0.1 mg/l NAA and 2 mg/l BAP showing callusing. **F,** Cultures under dark incubation on MS medium containing 0.1 mg/l NAA + 0.1 mg/l BAP (left), 0.1 mg/l NAA + 0.3 mg/l BAP (middle) and 0.1 mg/l NAA + 0.5 mg/l BAP (right) showing enhanced shoot growth and multiple shoots.

Table 1 *Effect of BAP and NAA on shoot-tip culture of Dolichos biflorus*

		Growth response			
BAP (mg/l)	NAA (mg/l)	Callus	Growth of shoot apex	Multiple shoots at nodal region	Rhizogenesis
—	—	—	+	—	+ +
—	2.0	+ + + +	—	—	+
0.1	0.1	+	+ +	+ +	—
0.3	0.1	+	+ + + +	+ + + +	—
0.5	0.1	+ +	+ + +	+ + +	—
0.7	0.1	+ + +	+ +	+ +	—
1.0	0.1	+ + + +	+	+ +	—
2.0	0.1	+ + + +	—	—	—

—, No response; +, very poor; + +, poor; + + +, good; + + + +, excellent.

the base of shoot-tip explants failed to show any morphogenetic response in subcultures.

The present work indicates the regenerative potential of shoot tips, which can be exploited to get virus-free clones by micropropagation.

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HISTOGENESIS OF A MOTH GALL ON *TEPHROSIA PURPUREA* (L.) PERS. (FABACEAE)

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THE stem galls, compared to the foliar galls, are usually simple in external form, but more complex internally. The higher level of structural complexity of the stem galls is due to involvement of more tissue types, especially, in certain cases, the vascular cambium, which is endowed with a high degree of histogenetic potential. Compared to the large number of stem galls so far recorded from India[1], only a few have been studied histologically[2,3]. The present paper deals with the histogenetic events in the formation of the stem gall on *Tephrosia purpurea* (L.) Pers. (Fabaceae) caused by a moth, *Dactylethra* *candida* (Staint)[3]. The growth of the gall involves bidirectional activity of a *de novo* meristem induced by the feeding activity of the moth.

The galls on *T. purpurea* occur mostly at the apices of young axes of axillary buds, sometimes of the main shoots, and rarely of the peduncle of the raceme. The number of galls per plant ranges from a few to more than twenty-five. Different developmental stages of the gall may be seen on a single plant. The mature galls are pyriform or flask-shaped (figure 1), greenish-pink or greenish-yellow, villous when young, glabrous or sparsely hairy at maturity. A fully grown gall, especially one from which the insect has escaped, is hollow, hard, and with a circular, fairly wide exit hole at the summit of the gall. Three or four consecutive internodes of a young shoot are involved in the formation of a gall and each gall measures approximately 1.2 cm in length and 8 mm in diameter. Malformed leaves persist on the gall (figure 1). The larval cavity is wide, single and axially oriented. The gall is somewhat circular in cross-sectional outline in contrast to the angular outline of normal stem (figures 3–5).

Examination of early stages of the gall indicates that the larva enters the young stem through the third or fourth internode of the shoot and reaches the pith, where it establishes a chamber by eating the cells around itself (figures 2 and 4). The initial responses of the cells bordering the larval chamber are somewhat similar to the process of wound healing, and involve dilation of cells. Outer to this zone, a few layers of cells become meristematic. The cells of this meristem divide, mostly in the periclinal plane and occasionally in the anticlinal plane as well. Though this meristem is aetiologically a wound meristem, by the bidirectional activity and additive mode of growth, it simulates cork cambium or vascular cambium (figures 5 and 7). The centripetal derivatives of the meristem differentiate into large thin-walled cells serving as a nutritive source to the larva. The centrifugal derivatives actually contribute to the growth of the gall and form a broad zone. As the cells of this zone mature, they develop fairly thick walls with simple pits (figure 6). At several loci of this zone groups of cells differentiate into amphivasal type of vascular nodules in which a few phloem elements are ensheathed by tracheary elements of isodiametric shape (figure 8). These elements resemble the neighbouring parenchyma cells, because the ground parenchyma cells differentiate directly and metaplastically into xylem elements.

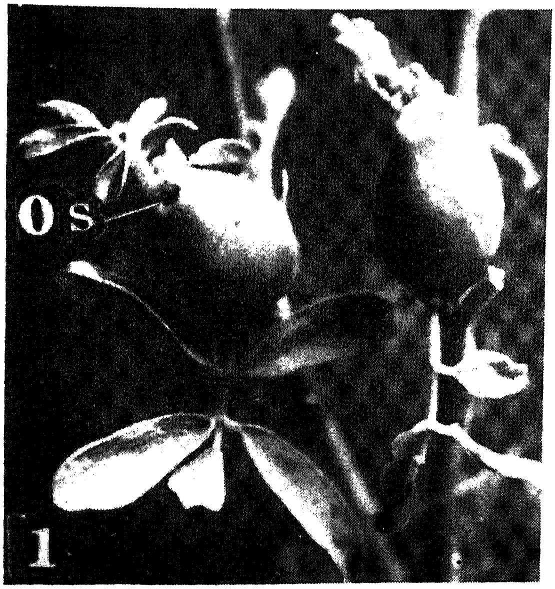
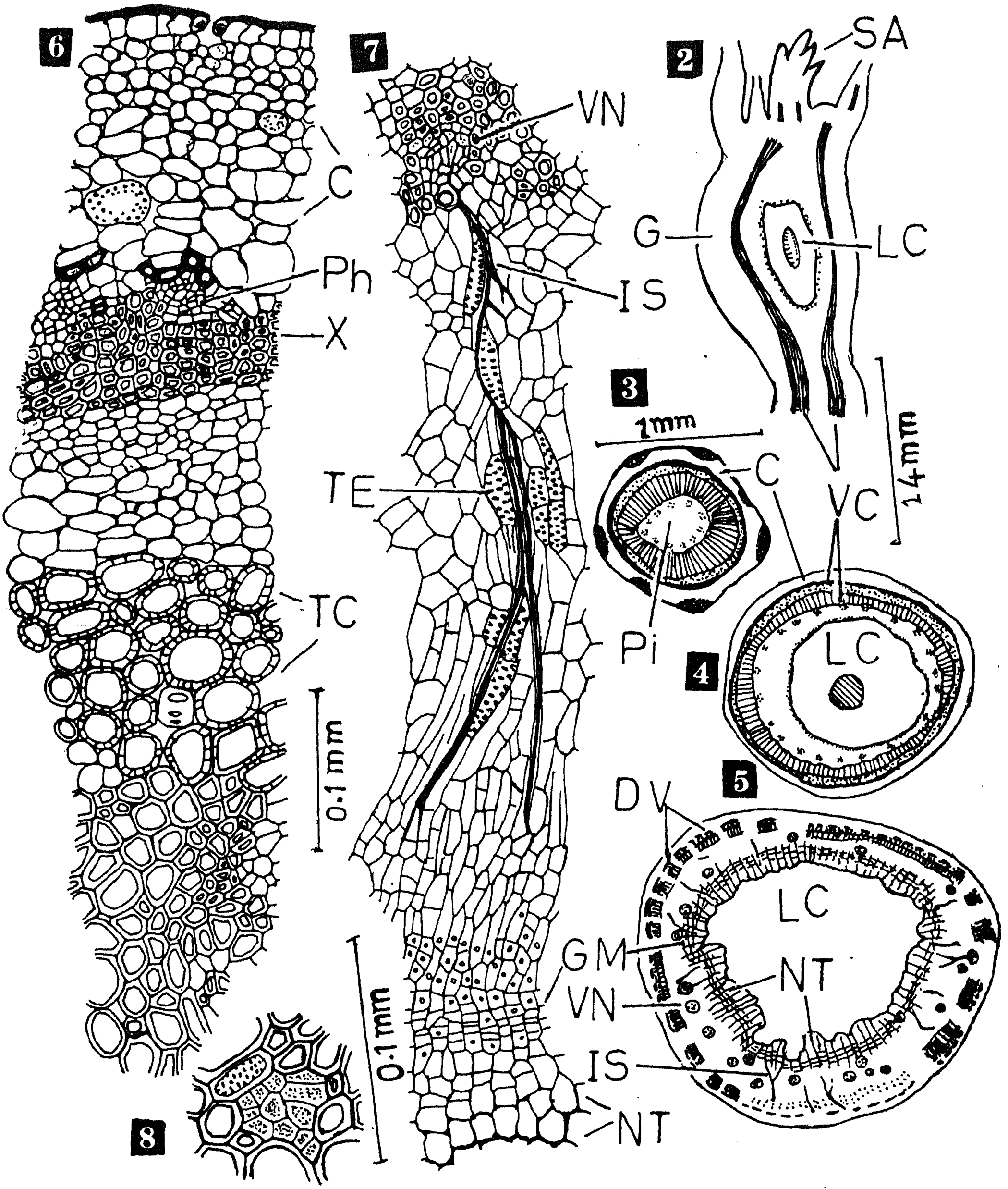


Figure 1. *Tephrosia purpurea.* A shoot bearing axillary bud galls, one of which shows a wide apical ostiole (os).

As soon as larval activity begins in the third or fourth internodal portion, the apical meristem of the affected shoot shows retarded growth, and internodal elongation is also reduced considerably, leading to condensation of the shoot system. The leaf primordia also do not attain the full stature of normal leaves (figure 1).

The vascular cambium of the axis ceases its activity soon after the onset of cecidogenesis. The vascular tissues formed up to then occur in the form of a narrow cylinder. Due to intrusion of the proliferating gall parenchyma, the vascular cylinder is cleaved into discontinuous sectors (figure 5). The xylem tissue has reduced dimensions of the elements and a low frequency of vessels compared to those of normal xylem tissue.

During growth of the gall, narrow radial strands of vascular elements differentiate from the parenchyma cells derived from the gall meristem. Each such strand evidently starts from the phloem elements of the amphivasal nodule or from the secondary phloem of the vascular cylinder (figures 5 and 7). They are simple or sometimes branched, and run into the nutritive tissue zone ending just outside the larval chamber. The central core of the strand contains cells with dark, amorphous substance and the ensheathing cells are short, pitted xylem elements (figure 7). Since these strands evidently



Figures 2–8. *Tephrosia purpurea.* **2,** L.S. of young gall; **3,** T.S. of stem; **4,** T.S. of young gall; **5,** T.S. of mature gall; **6,** Cellular detail of outer sector of gall; **7,** Inner sector of gall; **8,** Amphivasal nodule with central phloem surrounded by xylem. C, Cortex; DV, divided vascular cylinder; G, gall; GM, gall meristem; IS, irrigating strand; LC, larval chamber; NT, nutritive tissue; Pi, pith; Ph, phloem; SA, shoot apex; TC, thick-walled cells; TE, tracheary elements; VC, vascular cylinder; VN, vascular nodule; X, xylem.

drain off nutrients from the vascular tissues into the nutritive zone of the gall, they are referred to as irrigating strands (*faisceaux d'irrigation*) in cecidological literature[4]. After the larva develops into an adult moth, it escapes by biting a circular hole at the apex of the gall (figure 1). After the exit of the insect, the gall cavity may be occupied by ants, fungal mycelium, or other insects for shelter.

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OCCURRENCE OF THE NEW SPECIES *AZOSPIRILLUM HALOPRAEFERENS* IN ASSOCIATION WITH RICE ROOTS

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BACTERIA of the genus *Azospirillum* are widely distributed in the rhizosphere of tropical and subtropical grasses[1]. Association of *Azospirillum* with the roots of rice plants has been reported[2]. It is generally accepted that *Azospirillum* can enhance the growth of the plant[3]. Initially two species of *Azospirillum*, viz. *A. lipoferum* and *A. brasilense* were reported[4], to which a third species, *A. amazonense*, was added[5]. A fourth species, *A. halopraeferens*, which is characteristic for its high salt (3% NaCl) tolerance, has been reported[6]. The occurrence of a variant of *A. halopraeferens* associated with rice roots is reported here.

Roots of a local rice variety were collected from a rice soil of pH 8.0. Root bits (0.5 cm) from the washed root system were inoculated in nitrogen-free semi-solid malate medium[7]. Undulating subsurface pellicle formed on incubation was purified by three serial transfers into fresh semisolid medium. Each transfer was made at 48 h.

The isolate was gram-negative, highly motile and vibrioid. It can grow and fix nitrogen in presence of 5% NaCl in the N-free semisolid malate medium, but it is not obligately halophilic. The characteristics of *A. halopraeferens* listed by Reinhold *et al.*[6], except DNA base composition, were compared with our isolate (table 1). Photomigrographs of our isolate, *A. lipoferum* and *A. brasilense* are shown in figure 1.

Based on the observations, it is concluded that our isolate is a nutritional variant of *A. halopraeferens* reported by Reinhold *et al.*[6] This variant has been deposited in the culture bank of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University.

With the occurrence of large areas of saline soils in the country and saline irrigation water, this highly

Table 1 *Characters of A. halopraeferens and present isolate*

Character	*A. halopraeferens*	Our isolate
Salt tolerance (NaCl)	3%	5%
Utilization of glucose	−	+
Utilization of sucrose	−	+
Acidification of peptone-based glucose broth	−	+

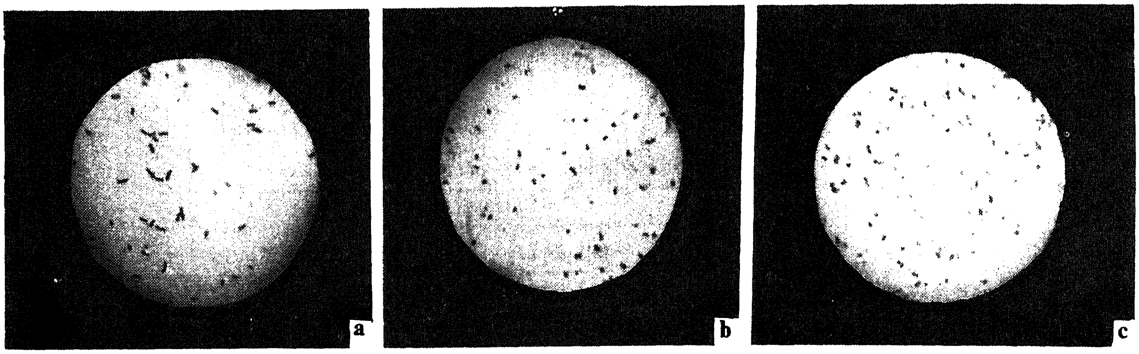

Figure 1. Photomicrographs of; **a,** *Azospirillum brasilense*; **b,** *A. lipoferum*; **c,** *A. halopraeferens*.

saline-tolerant diazotrophic *Azospirillum* might be interesting for practical applications.

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BOVINE MANURE LIQUOR AS A GROWTH MEDIUM FOR *RHIZOBIUM* INOCULANTS

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WE examined if bovine manure liquor could serve as a substitute for yeast extract mannitol broth to support the growth of rhizobia.

Fresh bovine manure collected from Jersey Cross breeds was used. The chemical composition of the oven-dried (45°C) manure was determined by standard procedures[1]. The manure contained 1.95% N (micro-Kjeldahl method), 0.71% P (vanadomolybdate yellow colour method), 0.3% each of Na and K (flame photometry), 4.4% Ca and 0.84% Mg (EDTA method), 0.149% of Fe, 0.023% Mn, 0.003% Cu, 0.013% Zn (atomic absorption spectrometry).

A 10% slurry in distilled water was prepared and filtered through muslin. The pH adjusted to 7.0 with 10 N HCl. The slurry was dispensed (100 ml) into 250 ml Erlenmeyer flasks. A set of flasks containing 100 ml of standard yeast mannitol broth[2] were also prepared. The flasks were autoclaved at 15 psi for 15 min. Another set of flasks containing 100 ml of 10% manure extract were also used without sterilization. All the flasks were inoculated with 1 ml of a 48-h-old *Rhizobium* culture (strain A1, a streptomycin-resistant strain from groundnut) and incubated at 28 ± 2°C for 7 days. Viable cell counts were made by dilution plate on congo-red yeast mannitol agar supplemented with 10 μg/ml streptomycin sulphate every 24 h for 7 days.

The results are presented in table 1. As expected, it was found that multiplication of *Rhizobium* in manure liquor was as good as in the commonly used but expensive yeast mannitol broth. The percentage increase in growth in yeast mannitol broth over that in sterile manure liquor was 6–21%, which is apparently not commensurate with the cost involved. On the other hand bovine manure liquor is absolutely cost-free to the farmer and is available without any seasonal limitations. But it should be sterilized before use since unsterilized manure liquor seems to inhibit introduced bacteria with a weak saprophytic ability either by metabolic inhibition or by exhausting nutrients needed for transient bacteria[3].

Table 1 *Multiplication of Rhizobium sp. in bovine manure liquor*

	Cell number ($\times 10^9$/ml)					
Treatment	Initial ($\times 10^5$/ml)	24 h	48 h	72 h	144 h	168 h
Yeast mannitol broth	0.39	00.04	00.32	46.70	49.00	43.70
Sterilized manure liquor	0.39	00.04	00.30	39.00	40.50	37.30
Unsterilized manure liquor	0.39	00.01	00.03	00.40	03.60	02.60

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EFFECT OF QUINONE EXTRACTED FROM *PYXINE PEFRICOLA* NYL. ON MITOSIS IN *ALLIUM CEPA* L. ROOT MERISTEMS

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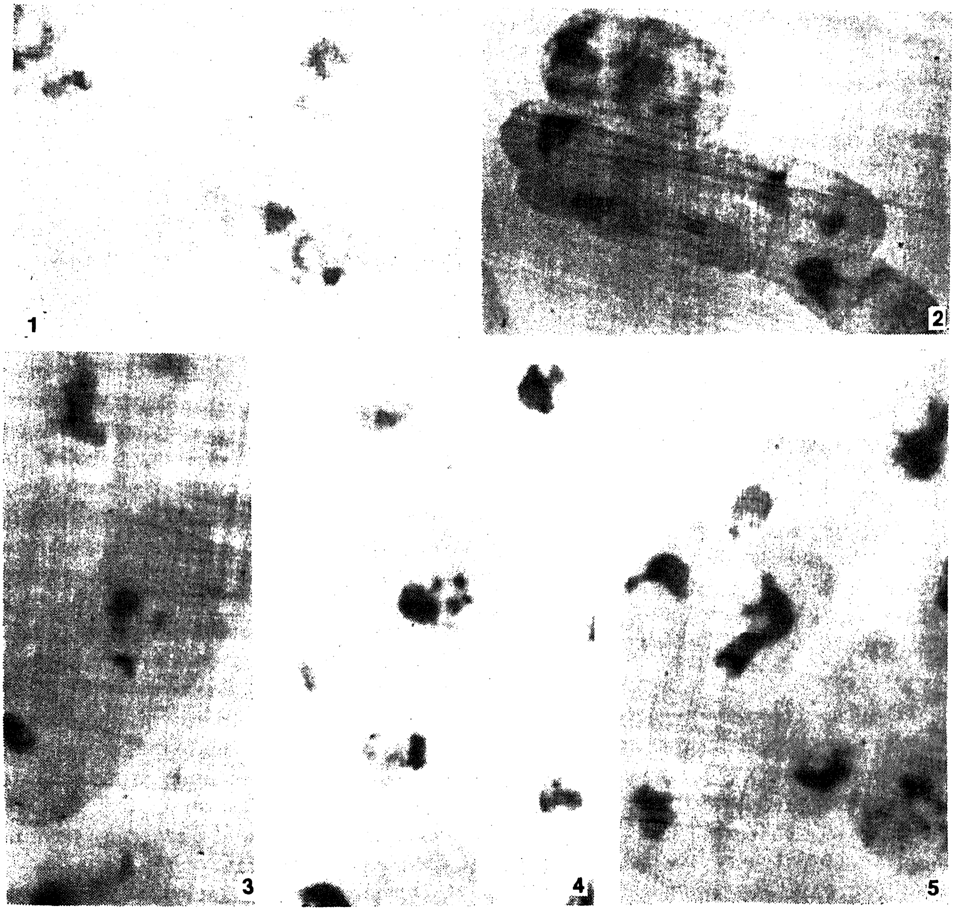
EXTRACTS of lichens are known to exhibit antibiotic[1], fungistatic[2] and mitosis-inhibitory[3-5] properties. In the present work an attempt has been made to study mutagenic effects of a newly synthesized and



Figures 1–5. Chromosome aberrations induced by quinone compound extracted from *Pyxine pefricola* Nyl. in root tip cells of *Allium cepa* L. (×675). **1,** Laggards and sticky chromosomes at telophase; **2,** bipolar unequal nuclei; **3,** tripolar unequal nucleus; **4,** stickiness and clumped chromosomes; **5,** nuclear stickiness causing deformation in nuclei.

identified quinone compound, extracted for the first time from the lichen species *Pyxine pefricola* Nyl., following the method described earlier[1,5,6]. In squash preparations the authors noticed some chromosomal aberrations and mitosis-inhibitory action of the compound.

Germinating bulbs of *Allium cepa* L. with 2-3-cm-long roots were kept in 0.01, 0.02, 0.05 and 0.1% of the compound in 1% aqueous NaOH (cytological study was made in alkaline medium as the compound was insoluble in water) for 6 h at 28 ± 2°C. Controls, in 1% NaOH solution, were also kept. After the treatment the root tips were fixed in a mixture of absolute alcohol and acetic acid (3:1), hydrolysed in N HCl at 60°C for 3–5 min, and squashed in haematoxylin. Nearly 10,000 cells (dividing and non-dividing) were screened in each experiment. In every slide the numbers of cells in prophase, metaphase, anaphase and telophase, and aberrant phases were counted. Mitotic index (MI) was calculated on the basis of number of dividing cells per 100 observed, while phase indices were calculated on the basis of ratio of number of cells observed in a given phase to total number of dividing cells. The χ^2 test was employed to find the significance of differences between control and treated plants.

The effect of the compound on cell division can be recognized by its reduction of MI and inhibition of anaphase. The decline in MI (except at 0.02%) is concentration-dependent (MI values: control (1% NaOH), 7.837; 0.01% quinone compound, 6.793; 0.02%, 7.228; 0.05%, 4.174 and 0.1%, 2.685). The proportions of cells in various phases were determined and the data suggest that the compound causes concentration-dependent inhibition of anaphase (anaphase index values: control, 162.116; 0.01%, 121.321; 0.02%, 91.425; 0.05%, 27.227 and 0.1%, 4.978).

Laggards and sticky chromosomes, bipolar and tripolar unequal sister nuclei, stickiness and clumping of nuclei and chromosomes (figures 1–5), bridges, split spindles, and c-mitotic cells and binucleate cells were also noticed. Root tips treated with 1% NaOH solution (control) showed very few aberrations. However, total per cent aberrations varied with concentration of the compound (per cent chromosome aberration: control, 0.910; 0.01%, 3.117; 0.02%, 4.959; 0.05%, 6.538 and 0.1%, 5.255). MI and per cent chromosome aberrations at 0.05 and 0.1% concentrations were significantly different.

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COMPETITIVENESS OF HOMOLOGOUS AND HETEROLOGOUS *RHIZOBIUM* FOR NODULATION AND GROWTH OF PIGEONPEA [*CAJANUS CAJAN* (L.) MILLSP]

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CULTIVATED soils in many parts of India are known to contain diverse groups of rhizobia that infect and produce nodules on a large number of species of the family Leguminosae. Thus a legume growing in such a soil is nodulated by all those rhizobia that can infect the host regardless of their effectiveness, unless the host is highly specific in its *Rhizobium* requirements. Besides, high competitive ability of *Rhizobium* is an important criterion for strain selection[1].

Failure of inoculation response could be due to either the presence of adequate population of efficient native rhizobia capable of nodulating the legume or failure of the inoculant strain to compete out native inefficient strains[2]. The present experiment was conducted to study the competitiveness of a homologous (effective) strain in the presence of a heterologous (ineffective) strain and the effect on nodulation and growth of pigeonpea [*Cajanus cajan* (L.) Millsp], an important pulse crop.

Competitiveness of homologous *Rhizobium* strain UASB 722 in the presence of heterologous strain UASB 11 was studied using Leonard jar technique[3]. The experiment involved inoculating host plants with the two strains in various proportions: (i) Control (uninoculated), (ii) UASB 722 only, (iii)

Table 1 *Competitiveness of homologous (UASB 722) and heterologous (UASB 11) Rhizobium for nodulation of pigeonpea*

Treatment	No. of nodules/plant		Total no. of nodules	Nodules formed by UASB 722 (%)	Nodule dry wt (mg/plant)		Total nodule wt (mg/plant)	Weight of nodules formed by UASB 722 (%)
	UASB 722	UASB11			UASB 722	UASB 11		
Control	0 (1)	0 (1)	0	0	0 (1)	0 (1)	0	0
UASB 722	90 (9.53)*	0 (1)	90	100	372 (19.3)	0 (1)	372	100
UASB 11	0 (1)	32 (5.7)	32	0	0 (1)	138 (11.7)	138	0
UASB 722+UASB 11 (8:2)	48 (6.9)	14 (3.8)	62	77	212 (14.5)	80 (8.9)	292	77
UASB 722+UASB 11 (6:4)	31 (5.6)	23 (4.8)	54	57	212 (14.5)	58 (7.6)	270	78
UASB 722+UASB 11 (5:5)	37 (6.1)	48 (6.9)	85	43	200 (14.1)	61 (7.8)	261	77
UASB 722+UASB 11 (4:6)	51 (7.2)	46 (6.8)	97	52	215 (14.6)	65 (8.1)	280	77
UASB 722+UASB 11 (2:8)	51 (7.2)	57 (7.6)	108	47	115 (10.7)	65 (8.1)	180	64

*Data in parentheses are transformation of $\sqrt{X}$.

	No. of nodules	Nodule wt
CD (5%)	0.17	0.28
SE	0.08	0.13
CV (%)	0.80	2.40

UASB 11 only, (iv) UASB 722+UASB 11 (8:2), (v) UASB 722+UASB 11 (6:4), (vi) UASB 722+UASB 11 (5:5), (vii) UASB 722+UASB 11 (4:6), (viii) UASB 722+UASB 11 (2:8). Each had four replications. Competitive ability was determined as per cent nodules formed by the two strains, which were typed on the basis of nodule morphology. Since the two strains formed morphologically distinguishable root nodules on pigeonpea, it was possible to identify the nodules by visual observation. The identification was confirmed by serotyping. Observations were made of nodule number, nodule dry weight, shoot dry weight, root dry weight and total biomass. The nodule number and nodule dry weight for each strain were recorded separately under mixed inoculation. The age of the crop at harvest was 50 days and the pigeonpea variety used was Hy-3C.

The nodules formed by UASB 11 were small and devoid of leghaemoglobin in contrast to the bigger, leghaemoglobin-containing nodules formed by UASB 722. The heterologous strain UASB 11 produced fewer nodules and lower nodule dry weight than the homologous strain UASB 722 in the single inoculation situations (table 1). With increasing proportion of UASB 11 (20–80%) in the mixed inoculum the number of nodules formed by it also increased from 14/plant to 57/plant, resulting in reduction in the infectivity of the homologous strain UASB 722 (77–47%). The nodule mass produced by UASB 722 was always higher compared to UASB 11 in both pure and mixed inoculations. However, nodule dry weight produced by UASB 722 was reduced in the presence of UASB 11. While the number of nodules produced by UASB 722 in mixed inoculation always decreased with decrease in its proportion in inoculum, the strain UASB 11 sometimes formed more nodules in mixed inoculations than in single inoculation. Total nodule dry weight was highest (372 mg/plant) in inoculation with only UASB 722. Total nodule dry weight formed by UASB 722 was reduced by 36% when UASB 722 was only 20% in a mixed inoculation.

Maximum plant dry weight and shoot dry weight were recorded in plants inoculated with only UASB 722, while the plants inoculated with UASB 11 recorded the lowest shoot, root and total plant weight (table 2). In mixed inoculations, increasing proportion of UASB 11 decreased shoot, root and total plant dry weight, but the magnitude of decrease was smaller in comparison to the decrease of nodule number and nodule dry weight.

Most pulse crops grown in India are nodulated by rhizobia of the cowpea miscellany group including pigeonpea. Thus it is important to know the effect of nodulation by a nonhomologous *Rhizobium* on symbiotic N_2 fixation by effective and compatible strains. The soybean *Rhizobium* UASB 11 ineffectively nodulated pigeonpea (table 1). This further supports the view that cross-inoculation groups were not discrete and many cases of boundary jumping occurred between these groups[4]. Nodulation of pigeonpea by *Rhizobium japonicum* has been reported earlier[5]. The heterologous strain UASB 11 occupied proportionately fewer nodule sites than the homologous strain UASB 722. In Australia the introduced effective *Rhizobium* strain TA-1 dominated the ineffective native rhizobia in clover but not in sub-

Table 2 *Competitiveness of homologous (UASB 722) and heterologous (UASB 11) Rhizobium assessed by dry matter production in pigeonpea*

Treatment	Shoot wt (g/plant)	Increase in shoot wt due to UASB 722 over UASB 11 (%)	Root wt (g/plant)	Increase in root wt due to UASB 722 over UASB 11 (%)	Total plant wt (g/plant)	Increase in total plant wt due to UASB 722 over UASB 11 (%)
Control	3.39	—	2.47	—	5.86	—
UASB 11	1.05	—	0.50	—	1.55	—
UASB 722	3.91	272	2.42	384	6.13	295
UASB 722+UASB 11 (8:2)	3.05	190	1.22	144	4.25	174
UASB 722+UASB 11 (6:4)	2.01	91	1.92	284	3.93	153
UASB 722+UASB 11 (5:5)	2.61	148	1.40	184	4.01	158
UASB 722+UASB 11 (4:6)	2.20	109	1.31	162	3.51	126
UASB 722+UASB 11 (2:8)	2.06	96	1.04	108	3.10	100

	Shoot	Root	Total biomass
CD (5%)	0.17	0.12	0.26
SE	0.08	0.06	0.12
CV(%)	3.10	3.90	3.00

terraneum[6]. There are also reports where ineffective or less-effective rhizobia are known to be more competitive than effective rhizobia. In midwestern USA the ineffective *Rhizobium* USDA-128 is known to dominate over the effective *Rhizobium* USDA-110. The competitiveness and symbiotic effectiveness of homologous strain UASB 722 were markedly altered in the presence of heterologus *Rhizobium* UASB 11. These results suggest that nonhomologous (ineffective) nodulation could result in reduced N_2 fixation.

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VARIATION IN ANTHER CULTURE EFFICIENCY AMONG DONOR PLANT TILLERS IN RICE

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GROWTH conditions, in particular the physiological state of the donor plant at the time of anther excision, are known to affect anther callusing response in a large number of plant species including rice[1-4]. The present study was undertaken to examine callusing response and subsequent plant regeneration potential of the calli of anthers of different tillers of rice plants.

Young panicles from the main culm and primary, secondary and tertiary tillers of rice cultivar Wu-10B were withdrawn separately. In each case, samples of anthers were taken from different sites along the length of the panicle. Smear preparations of the anthers were stained with propionocarmine and examined microscopically to determine the developmental stage of the microspores. Anthers from florets of the region with microspores at mid-to-late-uninucleate stage were excised separately from different tillers and plated on N_6 medium[5] supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid and 30 g/l sucrose and gelled with 10 g/l agar. Immediately after inoculation, the cultures were subjected to a cold treatment of 8°C for 10 days in the dark. Then they were transferred to low light intensity of about 600 lux at 25 ± 1°C. Observations for visible callus induction were recorded till 60 days. For plant regeneration, the calli were transferred, after a week of their emergence from the anther lobes, to Murashige and Skoog's (MS) medium[6] supplemented with 1.0 mg/l kinetin and 0.5 mg/l 6-benzylaminopurine. Cultures showing shoot initiation were transferred from low light conditions to a regime of 4000 lux, 12 h photoperiod.

Ample variation for callusing and regeneration response was noticed in anthers from different tillers (table 1). Callusing response was the highest in anthers obtained from the main culm, and showed a steady decline in anther cultures from primary, secondary and tertiary tillers, in that order. However, regeneration frequency, calculated on the basis of calli subcultured, did not differ much for calli from the main culm, primary and secondary tillers, but was very low for calli from tertiary tillers.

Table 1 *Anther culture efficiency of different donor tillers of rice cultivar Wu-10B*

	Anthers			Calli		Regeneration frequency	
Donor tiller	Number inoculated	Number showing callus	Callusing frequency (%)	Number subcultured	Number regenerating	on the basis of calli subcultured (%)	on the basis of anthers inoculated (%)
Main culm	685	168	(24.5)	168	42	(23.2)	(6.13)
Primary	1065	131	(12.3)	131	36	(27.5)	(3.38)
Secondary	1700	151	(8.9)	151	47	(31.1)	(2.76)
Tertiary	1370	21	(1.5)	21	3	(14.3)	(0.22)

Thus, while callus induction frequency showed a distinct drop from main culm to tertiary tillers, there was no corresponding decline in regeneration response except in the case of tertiary tillers where both callusing and regeneration response were very low. The overall culture efficiency, worked out on the basis of anthers inoculated, was the highest for main culm and lowest for tertiary tillers.

These results indicate that the main culm is the most suitable source of anthers for high culture efficiency; primary and secondary tillers are likely to give appreciably lower response, while tertiary tillers are perhaps unsuitable for anther culture. It seems that the anthers drawn from the main culm offer a physiological state that is more amenable to *in vitro* manipulations. Further investigations leading to the understanding of the causes of this variation in response should afford some manoeuvrability for improvement of anther culture efficiency not only in rice but in many other systems as well.

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INFLUENCE OF N AND P ON CADMIUM TOXICITY ON PHOTOSYNTHESIS IN RICE

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CADMIUM, a non-essential metal, is considered potentially hazardous to plants and animals[1]. Rock phosphate constitutes one of the sources of Cd pollution. It is reported[2] that Cd content of P fertilizers varies from 2 to 200 ppm. Effluents from fertilizer industries contain Cd along with high levels of urea-N and P as pollutants. Photosynthetic ability of green plants provides an important physiological criterion to assess pollution stress. Photosynthetic ability of two physiologically different rice cultivars under conditions of cadmium toxicity was studied.

Twentyfive-day-old seedlings of the high-yielding semidwarf IR36 and the photosynthetically efficient, tall Swarnaprabha of identical duration were used for the study. Two seedlings of each cultivar were transplanted to nutrient culture solution[3] contained in 5 l capacity porcelain pots. The various treatments, viz. 3 ppm Cd as $CdCl_2$, 150 ppm each of urea-N and P as KH_2PO_4, began after a week. Control pots received only nutrient solution. The culture solution was maintained at pH 4.5 and renewed once every week. The treatments were arranged in a factorial design with two replications. Photosynthetic rate of the intact leaf *per se*, photosynthetically active radiation and stomatal CO_2 conductance were measured using a portable IRGA (ADC, UK). Chlorophyll content[3] (*a*, *b* and total) was also determined. The investigations were carried out about 15 days prior to flowering.

Since all the observations were made under least variation of relative humidity (2–4%) and photosynthetically active radiation (8–15%), the two varieties were compared for each treatment independently.

Overall photosynthetic rate was found to be highest under urea + P treatment, followed by P alone, and lowest under U + P + Cd treatment (table 1). In general, the cultivar Swarnaprabha showed higher photosynthetic rate than IR36.

The deleterious effect of Cd on photosynthetic rate was quite evident because Cd alone was found to be impairing carbon assimilation, which was further aggravated when Cd was combined with P. The decrease under the latter treatment was 40% compared with control. The decrease in photosynthetic rate was about 19% in the case of Cd treatment, while it was only 7 to 8% in urea + Cd treatment. The deleterious effect of Cd was further aggravated when it was combined with either P or P + U, although the P + U treatment resulted in the highest photosynthetic rate in both varieties. Of the two varieties, Swarnaprabha was found to be more sensitive to Cd toxicity than IR36.

The reduction in photosynthetic rate was evident from the concurrent reduction in chlorophyll content under Cd or Cd + P treatments. Apparently, chl *a* was more adversely affected than chl *b* owing to Cd toxicity. Chl *b* molecules serve mainly as

Table 1 *Effects of high levels of N, P and Cd on rice physiology*

Treatment	Variety	RH (%)	PAR (μE m^{-2} sec^{-1})	Stomatal conductance (cm/sec)	Chlorophyll Total (mg/g)	Chlorophyll *a*/*b* ratio	photosynthetic rate (mg CO$_2$ dm^{-2} h^{-1})
Control	CV. 1	59	720	0.690	3.68	3.6	19.4
	CV. 2	58	750	0.561	2.92	3.8	17.7
Urea (U)	CV. 1	60	720	0.772	4.35	3.3	18.7
	CV. 2	50	680	0.665	3.65	4.3	16.1
Phosphorus (P)	CV. 1	57	695	0.618	4.01	3.9	20.8
	CV. 2	56	780	0.639	2.48	3.9	19.4
Cadmium (Cd)	CV. 1	52	760	0.525	2.67	2.7	15.4
	CV. 2	51	740	0.502	2.51	2.2	14.6
U+P	CV. 1	49	680	0.711	4.21	3.8	22.2
	CV. 2	52	710	0.666	3.90	3.2	18.8
U+Cd	CV. 1	60	810	0.642	2.82	2.9	18.4
	CV. 2	58	830	0.626	2.68	2.7	16.4
P+Cd	CV. 1	57	840	0.515	2.58	3.1	11.0
	CV. 2	56	790	0.510	2.32	3.0	11.3
U+P+Cd	CV. 1	57	820	0.505	2.33	3.1	10.1
	CV. 2	58	810	0.512	2.29	2.3	8.8
C.D. 5%							
Treatment (T)		3.2	24.3	0.04	0.62	0.4	2.1
Variety (V)		NS	NS	0.02	0.43	NS	1.7
T × V		2.6	34.3	NS	NS	NS	NS

CV. 1, Swarnaprabha; CV. 2, IR36.
PAR, Photosynthetically active radiation.
NS, Not significant.

antenna molecules more conspicuously engaged in harvesting photons and are less prone to adverse situations. But the degradation of chl *a* molecules is indicative of profound damage to the photosynthetic apparatus. Root and Miller[4] found that Cd-induced chlorosis might be due to changes in Fe:Zn ratios in corn leaves.

The correlation study revealed a close positive association of photosynthetic rate with stomatal conductance ($r=0.750$) and chlorophyll content ($r=0.631$). The rate of stomatal conductance (CO$_2$) is so closely associated with assimilation rate that it might be inferred that adversely acting treatments like Cd, P+Cd or U+P+Cd had some stress effects on the opening and closing of stomata, thus governing the rate of conductance of CO$_2$. Huang *et al.*[5] reported almost complete absence of CO$_2$ assimilation in soybean receiving 900 μM Cd in a sand culture experiment. Further, they also observed a depression in photosynthesis to the extent of 60% on the day of peak photosynthetic activity when the plant was subjected to 450 μM Cd treatment. Evidently, Swarnaprabha is more adaptable to such stress environments than IR36. The benefit of U+P treatment can be related to higher photosynthetic rate as well as higher chlorophyll content under this treatment.

Injury to plants due to exposure to heavy metals may arise from replacement of another metal in an enzyme, causing interference in a specific metabolic process[6]. The present study suggests that the inhibitory effect of Cd can be checked to some extent by urea but not by phosphorus.

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OCCURRENCE OF HITHERTO UNKNOWN INSECTS OF MAIZE IN INDIA

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This paper 'Occurrence of hitherto unknown insects of maize in India' by L.M.L. Mathur, M.C. Joshi and M. Arif, has been withdrawn by the authors. The intimation came to us after this issue was printed.

-Ed.

Decachaetophora aeneipes (de Meijere) (Diptera: Sepsidae)

Maize seeds damaged by the maggots was first noticed in heavily manured fields at Auli (Joshimath, 3000 MSL) during May–June. The eggs were laid on the soil and the newly hatched maggots made their way into the seed. The infested seeds either did not germinate at all or bore with weak seedlings which did not persist much longer. Preliminary observations had shown that the infestation due to these maggots varied from 7 to 15% in each row of a 6-row plot compared to those sown 10 and 20 days thereafter.

The full-grown maggot is cream-coloured and measures about 4.5 mm in length. The apodous worm has a pair of dark hook-like mandibles at its pointed end and the body segments are indistinct. The grey-bodied fly measures 5 mm in length. The second antennal articulation bears no angular projection, the metatarsi of hind legs are slender, the anal cell and lower cross-veins of the wing are present, the costa remains unbroken, and the metastigmatal bristles are present. Hypandrium and aedeagal apodemes are completely fused and the ovipositor is non-piercing type.

Agrotis c-nigrum Linnaeus (Lepidoptera: Noctuidae)

The cutworm remains active during May–June at high altitudes where summer maize is grown as food and fodder. As many as 6 larvae of variable size and growth were found associated with roots of each infested plant. As a consequence of their feeding on the root hair and primary rootlets of one-month-old seedlings, the plants either showed sickly appearance or lay on the earth surface. The infestation in a 10 × 3 m plot was about 32%.

The greasy, mature larvae measure 35 mm long and the general appearance of the body is red to olive-green. The head is red-brown, the lateral yellow bands along the spiracles are mixed with brown spots, and the transverse band at the junction of the third thoracic and first abdominal segments is more prominent. The medium-built adults are dark or red-brown and the wing expansion is almost 44 mm (ref. 2). The collar has whitish scales. Each forewing bears a double sub-basal and antemedial unevenly curved lines, triangular black patches before and after a pale triangular patch emerging from the middle costa, the post-medial line bears a series of dark specks, and the sub-marginal line is indistinct. The hind wings are slightly pale and their underside bears an indistinct post-medial line and a cell spot.

Popillia pulchrips Arrow (Coleoptera: Rutelidae)

The adults were seen feeding on the silk during September 1987 at Nagenahalli, Mysore. The extent of damage was such that not a single ear remained untouched by the insect in the locality. As a result of such feeding the emerging silk was badly damaged and the grain-filling was adversely affected.

The metallic green, blue or coppery adults measure 10–12 mm long and 6–7 mm broad[3]. The body is elongate-oval in shape, very smooth and shining with a small but compact tuft of greyish hair on each side of the pygidial base, and a thin clothing of hair on the underside of the body. The clypeus is rugose, forehead and pronotum finely punctured, and scutellum almost smooth. Each elytron bears a deep transverse impression behind the scutellum, a finely punctured striae, a much wider row of striations, and a last row of few punctures. The pygidium is coarsely transversely punctured and the mesosternal process is compressed, curved and almost blunt. The foretibia of the male is armed with two sharp teeth, the lower lobe of the inner front claw is not angulated, and the longer claw of the middle foot has no cleft.

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A NEW RECORD OF SEED SETTING IN SCENTED GERANIUM (*PELARGONIUM GRAVEOLENS* L. HERIT.) FROM INDIA

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Two viable seed-setting strains have been identified in scented geranium at the Indian Institute of Horticultural Research for the first time in India and are described here.

Pelargonium graveolens L. Herit, a native of Cape Province, South Africa, is the source of geranium oil, which is highly prized in the perfumery industry. The plant was first introduced in Shevroy hills of Tamil Nadu and is presently cultivated on a commercial scale in the hills and plains of South India. Though the species flowers profusely, the flowers are sterile and so far seed set has not been reported in India[1]. Due to inherent sexual sterility, the plant is propagated vegetatively by stem cuttings and hence lacks sufficient genetic variability, limiting the development of genotypes superior in oil yield and quality. As sexual reproduction in such species would create genetic variation through recombination, the germplasm maintained at IIHR, Bangalore (980 m MSL, 13°58′N and 78°E) was screened for fertile seed-bearing genotypes. In two clonal lines, viz. PG-7 (source: HRS, Kodaikanal) and Algerian-4n (IIHR selection), plants with flowers bearing fertile anthers were observed in April/May 1988. Frequency of fertile flowers was 2–3% per plant. Fertile anthers, 2 to 4 in number (out of a total of 7 anthers) were of bright orange colour, plumpy, and were borne on extended filaments (figure 1). This is in contrast to the pale yellow, shrivelled anthers borne on short filaments generally found in the sterile flowers. The anthers dehisced normally, releasing abundant, orange-coloured and stainable[2] pollen. Selfed flowers produced seeds. This is the first

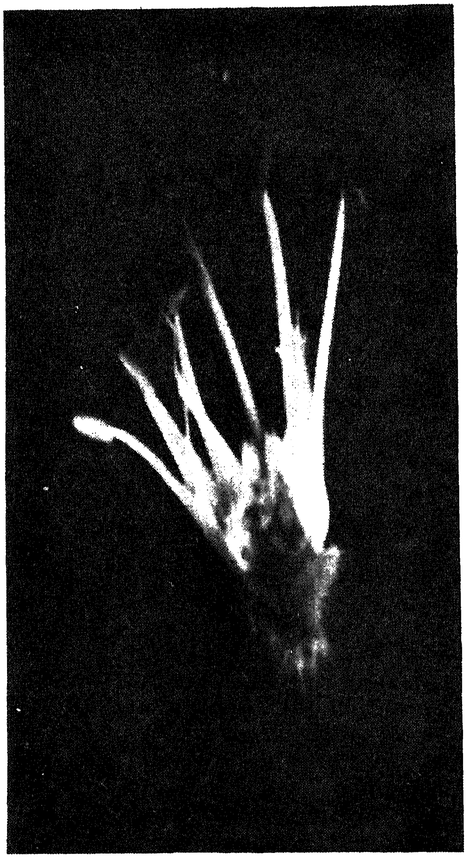

Figure 1.

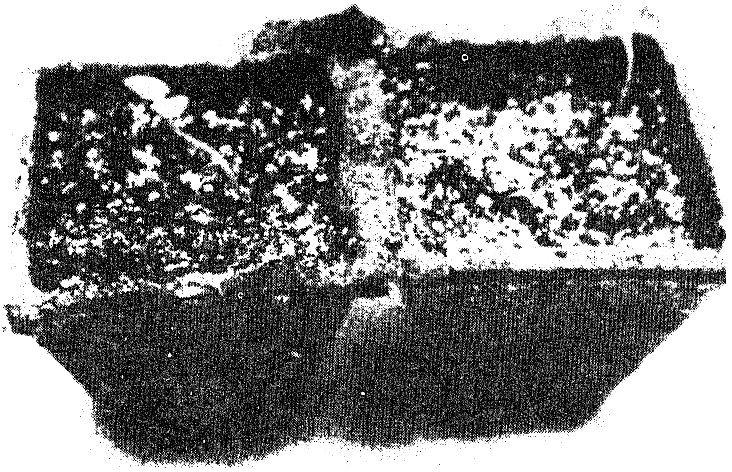

Figure 2.

record of seed set in scented geranium from India. Mature seeds oval in shape and dark brown to black in colour could be germinated in petri plates incubated in a seed germinator at 25 ± 1°C for 8–10 days. Seeds collected in June 1988, packed in polythene bags, and stored in a domestic refrigerator at 4°C remained viable for one year. Fresh mature seeds collected in June 1989 were also kept for germination, along with stored seeds. Good germination among fresh seeds was recorded, indicating that there is no seed dormancy. Seedlings from fresh and stored seeds were propagated in 4 cm pots containing vermiculite (figure 2) and surface-irrigated daily with a nutrient solution[3]. Initial growth of the seedlings was slow. After 45 days, the well established seedlings were transferred to sand + FYM mixture in large pots. Further studies on these plants are in progress.

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STAINING OF THE NEUROSECRETORY CELLS AND MATERIAL BY EISEN HAEMATOXYLINE

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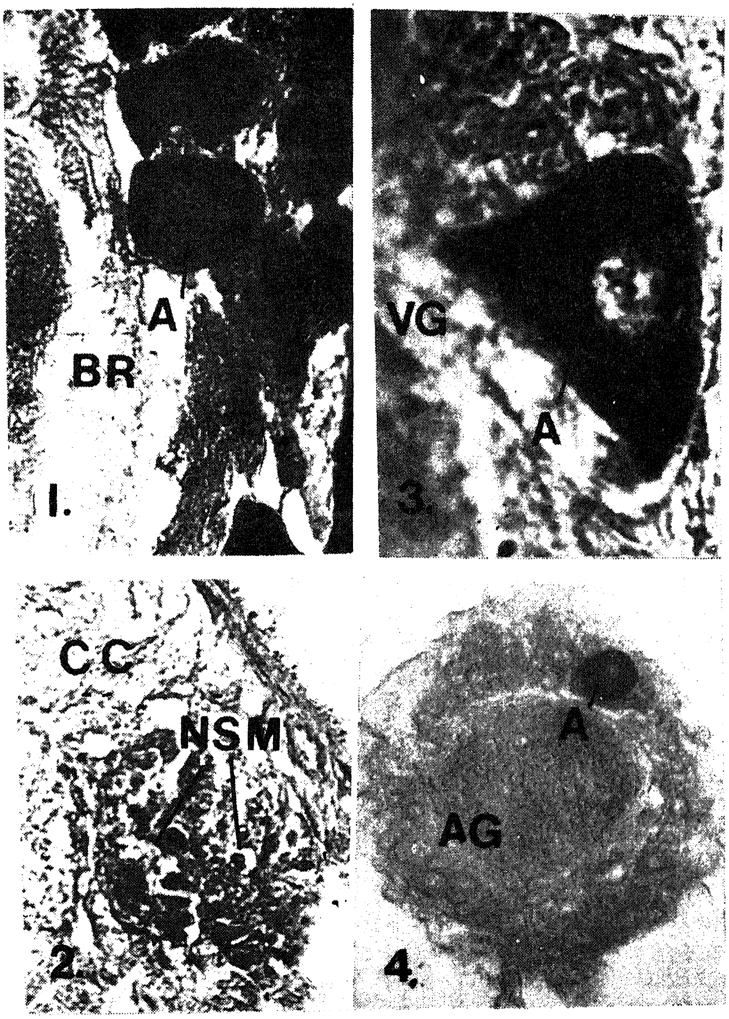
WEYER[1] was the first to describe neurosecretory cells in insects more than fifty years ago. The study of insect neurosecretion progressed slowly between 1930 and 1950. However, it gained momentum after the work of Gomori[2] and Gabe[3]. In the last twenty years several histochemical, electron-microscopic, radioautogrphic, immunohistochemical and other experimental methods were introduced[4-7]. Recently, Panov[8] reviewed different staining procedures used by different workers. However, so far there is no report of the use of Eisen haematoxyline for staining neurosecretory cells and material in insects. In this note it is shown that Eisen haematoxyline can be used as an alternative stain for neurosecretory cells and material in insects.

Larvae of *Galleria melonella* and adults of *Dysdercus cingulatus* were dissected in insect Ringer.

The brain, along with retrocerebral complex and ventral ganglionic chain, was removed and fixed in Bouin's fluid. Paraffin sections (5 μm) were cut. Stain preparation and staining procedures were as described earlier[9].

The A type neurosecretory cells in brain and thoracic and abdominal ganglia were stained dark blue (figures 1, 3 and 4). The B, C and D type neurosecretory cells were stained red with blue tinge with phloxine as counterstain (in the original staining eosin was used as counterstain). The nucleoli in the nucleus were stained red. The neurosecretory material in the axonic pathways and corpora cardiaca was stained as blue granules (figure 2).

The A cells identified by this technique were further confirmed with chrome alum haematoxyline phloxine[2] and aldehyde fuchsin[10], which are regarded as the best staining techniques for the identification of neurosecretory cells and material.



Figures 1–4. **1,** Neurosecretory cells of brain of *Dysdercus* (×400). **2,** Corpora cardiaca of *Dysdercus* showing neurosecretory material (×400). **3,** Neurosecretory cells of prothoracic ganglion of *Galleria* VII instar larva (×400). **4,** Neurosecretory cells in abdominal ganglion of *Galleria* VII instar larva (×100). A, (A type neurosecretory cells; AG, abdominal ganglion; BR, brain; CC, corpus cardiacum; NSM, neurosecretory material; VG, prothoracic ganglion.)

Thus the Eisen haematoxyline technique can also be used for staining neurosecretory cells and material in insects.

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RECORD OF *BEAUVERIA BASSIANA* (BALSANO) VUILLEMIN ON MEMBRACID SPP.

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ALBIZIA FALCATARIA, an exotic, being tried for afforestation programme in the states of Kerala, Karnataka and Tamil Nadu in view of its fast growth potential, was found attacked by two species of Membracidae, viz. *Oxyrachis* sp. and *Otinotus* sp., especially during the early years of its establishment. Saplings were debilitated (Pillai & Gopi, unpublished). During a survey of a one-year-old plantation for insect pests at Arippa (Kerala) in July 1986 (monsoon period), mass-scale mortality of membracids of the above species was noted. The affected insects became transformed into white mummies (figure 1). Of the 500 specimens collected in the field at random, 164 were dead with symptoms of fungal infection.

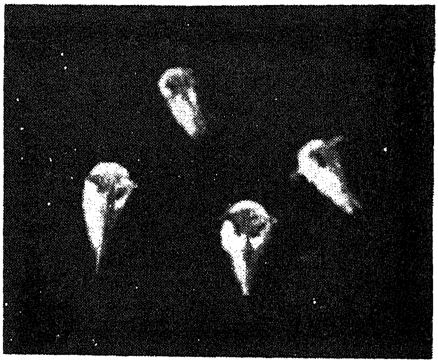

Figure 1. *Oxyrachis* sp. and *Otinotus* sp. parasitized by *Beauveria bassiana* (Bals.) Vuill.

The pathogen was isolated in the laboratory from the infected insects and cultured on potato dextrose agar. It was identified as *Beauveria bassiana* (Bals.) Vuill., commonly referred to as white muscardine fungus. Petri dish cultures initially appeared whitish and later turned chalky owing to prolific sporulation.

Pathogenicity of the fungus was tested by spraying 20 healthy nymphs and adults (10 each) with a concentrated suspension of spores. The insects were maintained under humid conditions at room temperature in rearing jars covered with muslin cloth. Control insects sprayed with sterile water were also kept under similar conditions. Within 4 to 6 days, all the inoculated insects were dead. These specimens later turned into white mummies with characteristic whitish growth of fungus over them.

Beauveria bassiana has been recorded as an entomogenous fungus of many insect pests of agricultural crops[1-5]. Recently Patil and Thontadarya[6] reported that this pathogen infects larvae of *Eutectona machaeralis*, a serious lepidopteran pest of teak, causing considerable mortality. The present record of this pathogen on a membracid, another pest of forestry importance, projects its potential as a biological control agent in forestry.

The authors are grateful to Dr M. A. J. Williams, Commonwealth Mycological Institute, England, for identifying the insect pathogen.

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A PIGMENTED, XYLOSE-UTILIZING STRAIN OF *STREPTOMYCES BOBILI*

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IN a preliminary screening of samples collected from special microsites in Agra, viz. mycorrhizosphere of pine; rhizosphere and rhizoplane of potato, gram, guar and chilly; and sewage and earthworm casts, for the isolation of antibiotic-producing actinomycetes, one isolate, J_4, was found to be strongly antagonistic to *Aschochyta rabiei*, which causes blight of gram, and *Alternaria alternata*, which causes leaf spot on chillies, papaya, sunflower, etc. The materials and methods used were similar to those of the previous communication and ISP procedures[1,2].

The screening of these isolates was done by placing the plugs cut from ten-day-old cultures of the actinomycetes in a petri plate previously seeded with test organisms.

The actinomycete isolate J_4 is a melanin-negative, chromogenic type producing soluble pigments of various shades on natural media. It shows strong amylase and tyrosinase reactions, but no cellulolytic, proteolytic or nitrate reduction activity. It is able to utilize glucose, sucrose, maltose, glycerol and xylose as carbon source. Mannitol, lactose and fructose were poorly utilized.

The vegetative mycelium is white, monopodially branched, forming compact growth in agar media. Aerial mycelium is abundant, white (1-A-7)[3] to pinkish-white (2-A-1)[3] turning to rosy pink (table 1). Sporophores are primitive spirals with open hooks (RA). Spores, measuring 3.4–3.3 × 3.3–1.6 μm, are in chains, oval to cylindrical, with smooth surface configuration.

Biological activity

The activity of the isolate J_4 against other microorganisms was tested by the streak method. The

Table 1 *Cultural characters of X J_4 isolate of Streptomyces bobili on various media*

Medium	Growth	Vegetative mycelium	Aerial mycelium	Reverse of colony	Remarks
Nutrient agar	Moderate	White	Pinkish white (49-B-1)	Mustard yellow to brown (14-K-9)	Dark brown soluble pigment
Tryptone yeast extract (ISP)	Excellent	White	Light pink (1-B-1)	Blackish brown (8-I-11)	Dark brown soluble pigment
Yeast malt extract	Good	White	Light pink (1-B-1)	Pinkish brown (7-J-11)	Maroon soluble pigment
Inorganic salt starch agar (ISP)	Excellent	White	Pinkish white (1-C-7)	Orangeish pink (2-E-8)	Strongly hydrolyses starch
Oat meal (ISP)	Excellent	Off-white	Pinkish white (1-C-7)	Pinkish brown (7-J-11)	Maroon soluble pigment
Glycerol asparagine (ISP)	Good	Dull white	Pink (49-C-1)	Light maroon (S-A-4)	Maroon soluble pigment
Melanin agar	Moderate	White	Pinkish white (10-A-1)	Light yellow (10-B-5)	Negative
Tyrosine agar	Good	White	Light pink (49-B-1)	Blackish brown (8-A-2)	Positive dark brown soluble pigment
Gelatin agar	Good	White	Light pink (9-A-1)	Rust orange (11-A-10)	Orange soluble pigment negative
PDA agar	Good	White	Pinkish white (49-B-1)	Violet (49-K-9)	Maroon soluble pigment

culture was inoculated by spore suspension as a broad streak about the edge of the petri plate on potato dextrose agar medium. After three days of incubation at 28 ± 2°C, the different microorganisms were streaked at right angles to the J_4 streak. The inhibition zone, if formed, was measured as a clear distance between the J_4 streak and the growth of the test organism.

J_4 was found to be active against *Alternaria brassicae, A. brassicicola, A. solani, A. raphni, Aspergillus niger, A. sulfureus, Fusarium solani, Curvularia lunata, Phoma palmarum, Penicillium janthenellum, Cladosporium sphaerospermum, Humicola fusco-atra, Botrydiplodia theobromae, Gleosporium candidum, Salmonella typhi, Pseudomonas pyocanea, Escherichia coli, Bacillus subtilis, Candida albicans, Staphylococcus aureus, Shigella* and *Proteus.*

Properties of antibiotic substance

The antibiotic produced by J_4 is thermolabile. With rise in temperature from 40 to 80°C the activity decreases gradually, and at temperatures above 80°C the antibiotic substance gets completely inactivated after 30 min.

On storage the activity is lost rapidly at 35–40°C. The antibiotic can be best stored without any appreciable loss in activity for up to 40 days at pH 7.0 at 7°C. The antibiotic is soluble in *n*-butanol, methanol, isopropyl alcohol, ethanol and ethyl acetate and moderately so in benzene, petroleum ether, toluene and chloroform. It was found to give a single spot in two-dimensional paper chromatography as well as by bio-autographic technique. The antibiotic contained only one component with R_f value 0.733 when the chromatogram was run in butanol : oxalic acid : H_2O (50 : 2.5 g : 50), and with R_f value 0.802 when the chromatogram was run in methanol : ethyl acetate (95 : 5).

The isolate J_4 was identified as a species of *Streptomyces* as its mycelium does not break up in bacilli or coccoid forms and instead forms a tough, textured mycelium.

The *Streptomyces* isolate described above was found to resemble *Streptomyces bobili*[4,5]. It produces active substance very similar to cinerubin produced by a known strain of *S. bobili.* However, it differs from the latter in the production of pigments of various shades in most natural media (table 1) and in the ability to utilize xylose. It has therefore been designated as a pigmented and xylose-utilizing strain of *S. bobili.*

The authors thank CSIR, New Delhi, for financial help.

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SCREENING FOR OLEAGINOUS YEASTS USING REPLICA PRINTING TECHNIQUE COUPLED WITH DENSITOMETRIC SCANNING

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MICROORGANISMS have been used as a source of protein and vitamins[1]. Yeast is commercially produced for animal feed[2]. Oleaginous yeasts are capable of accumulating oil within the cells[3]. Microbes can be exploited to produce fat. The advantages of using microbes are their rapid rates of growth and ability to accumulate fat using naturally occurring substrates. Thus single cell oil has recently acquired importance[4]. With this in view, we have screened selected environments for the presence of oleaginous yeast[5]. For screening a large number of isolates, routine methods like pure culture isolation and extraction of fat by chemical methods from every isolated culture are laborious and time-consuming. There is need for a quick screening method for picking up promising oleaginous strains.

The replica printing method was first reported by Evans *et al.*[6] to select high fat-accumulating mutants of *Candida curvata, Lipomyces starkeyi* and *Trichosporon cutaneum* produced by artificial mutagenesis.

Environments with high probability of isolating oleaginous yeasts were selected. Representative samples of different environments were obtained. About 120 samples were collected for screening from (i) industrial sewage from starch industries and breweries; (ii) dairy waste; (iii) bakery waste; (iv) soil from petrol filling stations and oil mills; (v) fruit waste from fruit juice and pulp industries; and (vi) oilseeds.

Yeast extract malt agar medium (YEMA) was used to plate the samples to isolate yeasts. Nitrogen-limited medium (NLM) with 2% agar as described earlier[7] was used to cultivate oleaginous yeasts.

Samples obtained were immediately processed and homogenized and ten-fold serial dilutions were prepared. They were plated on YEMA plates and incubated at 26°C for 72–96 h.

From the YEMA plates, colonies were replica-plated onto NLM agar plates in duplicate in the following manner. Nitrogen-limited agar plates were divided into squares and were numbered. Individual colonies were picked from the YEMA plates with sterilized wooden sticks and spotted onto the NLM agar plate, one in each square. These were then incubated for 72–96 h at 26°C.

An impression of the colonies on the NLM agar plates was made on filter paper (Whatman No. 1) by pressing it against the plate. The filter paper was dried under vacuum for 30 min at 50°C and stained for 20 min with Sudan Black B (0.08% w/v in 95% ethanol). Excess stain was removed by washing in 95% ethanol and destaining again in 95% ethanol for 5–10 min. The filter paper was finally air-dried[8].

A photovolt densitometer, model 52.CN.Y., was used. The densitometer is provided with a scanner that measures percentage absorption of the incident light (525 nm). The intensity of colour of colonies stained specifically for fat was recorded as peaks on a recording chart. Thus percentage absorption is a direct measure of the quantity of fat accumulated by the strain.

Lipid extraction was done chemically as described previously by Sobus and Holmlund[9].

Yeast strains

i) Different isolates of yeasts as samples; ii) *Trichosporon cutaneum* NCIM 3151 as positive control, high lipid-accumulating strain (lipid content was 40%); iii) *Saccharomyces cerevisiae* No. 3076 (NCIM, NCL, India) as negative control (lipid content was 10%).

Figure 1 shows the result of the staining of yeast strains isolated from different environments, along with a positive control and a negative control. Figure 2 shows a densitometric scan of stained colony impressions corresponding to figure 1. Per cent absorption is in direct proportion to the

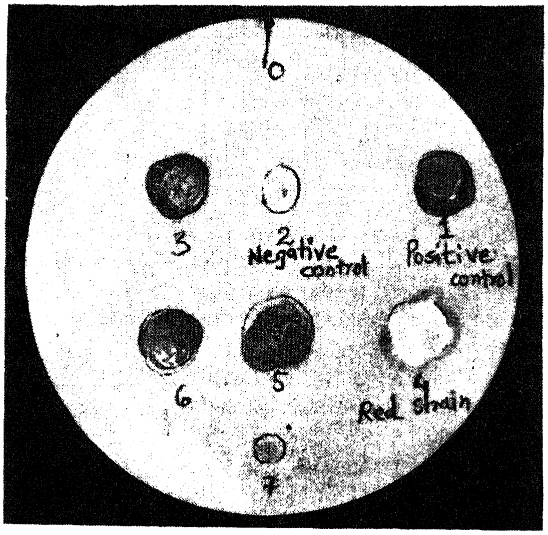

Figure 1. Yeast colony impressions after replica printing stained with Sudan Black B. **1,** *Trichosporon cutaneum*—positive control; **2,** *Saccharomyces cerevisiae*—negative control; **3,** *Endomyces*; **4,** *Rhodotorula rubra*; **5,** *Debaromyces hansenii*; **6,** *Hansenula jadinii*; **7,** *Candida kruseii*.

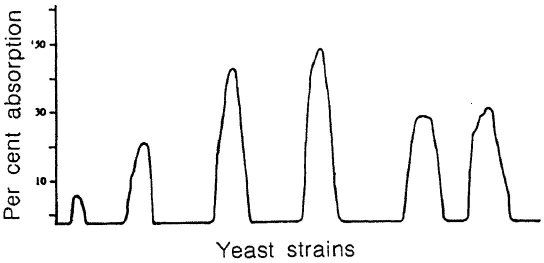

Figure 2. Densitometric scan of stained colony impressions from left to right: negative control *S. cerevisiae* NCIM 3076, positive control *T. cutaneum* NCIM 3151, *C. kruseii*, *Endomyces*, *D. hansenii*, *H. jadinii*.

Table 1 *Correlation between optical density of stained oleaginous yeasts after replica printing and fat yield*

Yeast strain	Fat staining after replica printing	Optical density at 525 nm of stained colonies	Fat yield (mg of fat/100 mg of dried biomass, carbon source glucose)
Trichosporon cutaneum NCIM 3151	+	45	40
Saccharomyces cerevisiae NCIM 3076	−	5	10
Endomyces	+	52	33 (55)*
Rhodotorula rubra	**	**	35
Debaromyces hansenii	+	33	27
Candida parapsilosis	+	38	29
Candida kruseii	+	22	23
Hansenula jadinii	+	33	27

Coefficient of correlation between optical density and fat yield +0.932.

*Carbon source xylose.

**Could not be detected.

intensity of colour development on the filter strip *Rhodotorula rubra* (red-pigmented) isolated from sewage was not stained after replica printing and was not scanned. It was seen that strains with less than 10% accumulated lipid were not stained and their per cent absorption was nil.

Intracellular lipid was extracted chemically from dried cell biomass. Fat was estimated gravimetrically as mg% fat. Table 1 shows the fat content of different yeast strains. It also shows that densitometric measurement of individual strains compares well with intracellular fat content (coefficient of correlation +0.932).

We have demonstrated that this method could be applied to selection of oleaginous microbes in samples containing a mixed group of microorganisms. Further we have quantitated the fat content of the strains by densitometric scan of stained colony impressions after replica printing. Thus, this method is as simple as the existing replica printing technique, and offers the advantage of simultaneous estimation of fat content. Colonies with high lipid content showed dark blue coloration and higher per cent absorption. Those with moderate lipid content showed light blue coloration after staining and lower absorption. Those with poor lipid accumulation could not be stained and gave no absorption. The oleaginous yeasts were cultivated on NLM as this medium enhances lipid accumulation. Even though the red-pigmented strain *R. rubra* accumulated lipid to as much as 35%, this technique could not be applied successfully to quantify the amount of fat accumulated by this strain as the red pigment produced by the strain interfered with the fat staining. Thus the present procedure has limitations in screening pigmented yeast strains.

The authors are grateful to Dr B. S. Narasinga Rao for his suggestions and keen interest.

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SERUM UREA AND ENZYMES IN RAT ON INTRAGASTRIC ADMINISTRATION OF GARLIC OIL AND GARLIC EXTRACTS

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HYPOGLYCEMIC, hypolipidemic, fibrinolytic and antibacterial activities of garlic principles are well known[1-8]. Garlic is generally considered as a safe flavouring adjuvant of foods. The recommended levels are 800–1300 ppm of garlic or 10–15 ppm of garlic oil[9]. However, very high doses of garlic principles are toxic. The LD_{50} value for allicin in mice is 60 mg per kg body wt[9]. Nakagawa *et al.*[10] observed that when young rats were fed a high dose of fresh garlic juice, some died within 21 days and the body wt of the still living ones was considerably reduced. Hypertrophy of the spleen, liver and adrenal glands, followed by decreased erythrocyte counts, were regular features in high-dose raw garlic-administered rats[10]. The active principles of aqueous extracts of garlic are sulphoxides; in garlic oil the active principle is diallyl disulphide[1]. Sulphoxides and disulphides can undergo exchange reactions with various sulphhydryl compounds in the body spontaneously at physiological pH and temperature[11,12]. Titrable SH groups are found on the cell membranes of RBC, and cells of liver and kidneys[11]. The diallyl disulphide of garlic oil is absorbed and metabolized chiefly in the liver[13]. We therefore studied liver and kidney function in rats administered high doses of garlic principles.

White male albino rats, 6–8 months old and weighing 200–300 g, were obtained from the Central Food Technological Research Institute, Mysore. They were divided into five groups of six animals each and fed standard laboratory diet *ad libitum*.

One gram of garlic was crushed with 3 ml water and filtered. The filtrate was used as aqueous extract of garlic. Crushed garlic was steam-distilled. The distillate was extracted with ether. The ether layer was separated, clarified with anhydrous sodium sulphate and evaporated at 55°C. The oil left behind was used as garlic oil.

Control rats (group 1) were fed intragastrically 2 ml normal saline per 100 g body wt 3 h before sacrifice. Group 2 rats were fed 2 ml aqueous extracts of garlic per 100 g body wt 3 h before sacrifice. Group 3 rats were fed 6.7 mg garlic oil suspended in 2 ml normal saline per 100 g body wt. Group 4 rats were fasted for 24 h and fed 2 ml normal saline per 100 g body wt and sacrificed 3 h later. Group 5 rats were also fasted for 24 h and fed 6.7 mg garlic oil suspended in 2 ml normal saline per 100 g body wt 3 h before sacrifice. All the rats were sacrificed by decapitation. Blood was collected in a clean beaker and allowed to clot. Urea[14], creatinine[15], LDH (EC 1.1.1.27)[16], alkaline phosphatase (EC 3.1.3.1)[17], AST (EC 2.6.1.10)[18] and ALT (EC 2.6.1.2)[18] were estimated in the clear serum. All the estimations were carried out using diagnostic reagent kits from SPAN Diagnostic Pvt. Ltd, Surat, India. *In vitro* addition of garlic extracts or garlic oil did not have any effect on the estimation of urea by the method used.

Statistical analysis was by Student's t test.

The results are shown in table 1. Group 2 rats showed significantly higher ($P < 0.01$) levels of urea and AST in serum, while ALT, alkaline phosphatase and LDH levels were unaffected. Feeding garlic oil to fed rats (group 3) did not change any of the above parameters within 3 h ($P > 0.1$). Rats fasted for 24 h (group 4) also showed only normal levels of urea, AST, ALT, alkaline phosphatase and LDH in serum. However, when 24-h-fasted rats were fed garlic oil (group 5) serum urea increased and alkaline phosphatase decreased significantly ($P < 0.01$). Administration of garlic extracts or garlic oil had no effect on serum creatinine.

Diallyl disulphide present in garlic oil and the sulphoxides present in aqueous extracts of garlic are known to undergo exchange reactions with SH groups of enzymes and other proteins that have titrable SH groups[11,12]. Thus garlic principles have been demonstrated to inhibit the activities of a variety of enzymes, including alkaline phosphatase[1]. The low levels of alkaline phosphatase in group 5 rats (fed garlic oil in the fasted state) may be due to such inhibition by garlic oil absorbed from the intestines and still present in the blood. Rats fed a similar quantity of garlic oil in the fed state (group 3) show no change in alkaline phosphatase probably because absorption of garlic oil might have been delayed.

Earlier workers have used a dose of 10 mg garlic oil or 1 ml garlic extracts per 100 g body weight of fed rats for hypolipidemic studies[5,6]. Yet administration of two-thirds of this dose of garlic oil to fasted rats raises urea and inhibits alkaline phosphatase in serum. Similarly administration of twice the optimal dose of garlic aqueous extracts to fed rats raises urea

Table 1 *Effects of feeding garlic principles on serum urea and enzymes in fed and starved albino rats*

Group	Treatment	Urea (mmol/l)	Creatinine (μmol/l)	LDH	Alkaline phosphatase	AST	ALT
1	Control	4.1 ± 0.38	127 ± 39	164 ± 23	376 ± 45	120 ± 20	32 ± 5.7
2	Garlic aqueous extracts	6.27* ± 1.05	169 ± 22	169 ± 15	387 ± 108	170* ± 21	38 ± 5.4
3	Garlic oil	4.05 ± 0.22	99 ± 54	148 ± 22	380 ± 29	142 ± 16	23 ± 5
4	24-h-fasted	4.0 ± 0.33	167 ± 22	175 ± 14.2	250 ± 24	130 ± 13	25 ± 2.6
5	24-h-fasted garlic oil	9.4* ± 1.3	132 ± 18	298 ± 259	15.5* ± 4.4	172 ± 51	43 ± 20

Results are mean ± SD for six observations.
*$P < 0.01$.

and AST levels within 3 h. Thus administration of an overdose of aqueous extracts of garlic to fed rats or a normal dose of garlic oil to fasted rats may cause changes in urea and enzymes in the serum.

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